Cardiomyocyte-Specific Overexpression of NO Synthase-3 Protects Against Myocardial Ischemia-Reperfusion Injury

John W. Elrod, James J.M. Greer, Nathan S. Bryan, Will Langston, Jeffrey F. Szot, Henock Gebregzlabher, Stefan Janssens, Martin Feelisch, David J. Lefer

Objective—The protective effect of NO synthase-3 (eNOS)–derived NO in limiting myocardial ischemia-reperfusion (MI-R) injury is well established. We reported previously that systemic genetic overexpression of eNOS attenuates MI-R injury. The purpose of the current study was to investigate tissue-specific genetic overexpression of the human eNOS gene.

Methods and Results—To accomplish this, we used 2 distinct murine models of transgenic overexpression, a cardiomyocyte-specific eNOS overexpresser (CS eNOS-Tg) under the control of the α-myosin heart chain promoter, and a systemic eNOS transgenic mouse (SYS eNOS-Tg) under control of the native eNOS promoter with an upstream endothelial enhancer element. Mice were subjected to 30 or 45 minutes of left coronary artery ischemia and 24 or 72 hours of reperfusion. CS eNOS-Tg mice displayed significantly decreased infarct size beyond that of mice with systemic overexpression. Additionally, CS eNOS-Tg mice exhibited better preservation of cardiac function compared with SYS eNOS-Tg mice after myocardial infarction.

Conclusion—These results provide evidence that site-specific targeting of eNOS gene therapy may be more advantageous in limiting MI-R injury and subsequent cardiac dysfunction. (Arterioscler Thromb Vasc Biol. 2006;26:1517-1523.)

Key Words: gene therapy ■ myocardial infarction ■ eNOS ■ cardiomyocyte ■ endothelial ■ cardiac function ■ blood pressure

Nitric oxide (NO) is a free-radical species generated by the enzyme endothelial NO synthase (eNOS). NO has been shown to play a prominent role in the maintenance of vascular homeostasis by acting as a vasodilator,1–3 attenuating platelet4 and leukocyte5 adhesion, inhibiting vascular smooth muscle cell proliferation,6 and possessing multiple other anti-inflammatory properties. eNOS is also present and functional in cardiac myocytes,7 in which NO generation has been shown8–11 to modulate cardiac function.

It is now well appreciated that NO can exert many beneficial effects in various cardiovascular disease states. NO has been shown to protect against myocardial ischemia-reperfusion (MI-R) injury in a number of animal models.12–14 These basic science observations have led to the recent translation of NO-based therapies to the clinic. The African American Heart Failure Trial found isosorbide dinitrate-hydralazine to significantly lower mortality rates in patients with class III and IV heart failure, prompting an early termination of the trial and introduction of isosorbide dinitrate-hydralazine (BiDil) to the medicinal armament.15 Additionally, a recent study of inhaled NO demonstrated acute hemodynamic improvements in patients with right ventricular myocardial infarction (MI) and cardiogenic shock.16 In addition to direct NO therapy, the role of NO synthase-3 (eNOS) in MI-R has been studied extensively. Mice deficient in eNOS (eNOS−/−) have increased myocardial injury after I-R, displaying significantly larger infarct size.17,18 Conversely, augmenting eNOS function has proven to be very effective at reducing MI-R injury. Supplementation of the eNOS substrate L-arginine, has been shown19,20 to reduce infarct size after MI-R injury. We reported previously21 the protective effects of systemic, genetic eNOS overexpression in MI-R. Others have reported that eNOS overexpression limited to cardiac myocytes provided increased left ventricle (LV) performance and reduced hypertrophy in a murine heart failure model.22 Given the protective nature of eNOS gene therapy in MI-R injury, we sought to compare cardiomyocyte-specific eNOS transgenic overexpression (CS eNOS-Tg) versus systemic transgenic overexpression of eNOS (SYS eNOS-Tg) in an in vivo murine model of MI-R injury. Both mice overexpress the same human eNOS transgene23 on the same background strain (C57BL/6). However, each transgene is under the control of different promoters. The transgene in the systemic overexpresser is under the control of the native eNOS
promoter,24,25 whereas the cardiomyocyte-specific transgene is driven by the α-myosin heavy chain (α-MHC) promoter,22 thus restricting expression to cardiac myocytes. These model systems of eNOS overexpression provide a powerful tool for the investigation of tissue-specific gene therapy in MI-R injury and help to further our understanding of targeted gene therapy.

Materials and Methods

SYS eNOS-Tg Mice

The systemic eNOS transgenic mouse (eNOS-Tg) was developed in Rotterdam and has been described previously.21 The mouse uses a human transgene20 driven by the native eNOS promoter.

CS eNOS-Tg Mice

CS eNOS-Tg mice were developed by S.J. using the same human eNOS transgene23 as the SYS eNOS-Tg mouse. The expression of this transgene is under the control of the α-MHC promoter, restricting expression to cardiac myocytes as has been described previously.22

Wild-Type Mice

Nontransgenic, wild-type (WT; C57BL6/L) littermates from both colonies of transgenic mice were randomized and used as controls. All mice were male and used at 8 to 10 weeks of age. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. [National Institutes of Health] 86-23, revised 1985. Animal Resources Program, DRR/NIH, Bethesda, Md), approved by the Council of the American Physiological Society, and with federal and state regulations. All experimental procedures were approved by the Louisiana State University Medical Center Animal Care and Use Committee.

Western Blot Analysis of eNOS

Western blot analysis was performed as described previously. Myocardial lysate was transferred to polyvinylidene fluoride membranes and incubated with mouse anti-eNOS (1:4000; BD Transduction Laboratories) in 5% BSA Tris-buffered saline Tween-20 (TBST) overnight at 4°C. Membranes were then reacted with horseradish peroxidase–linked anti-mouse secondary (Amersham) at 1:2000 in 5% BSA TBST, incubated with ECL reagents (Amer sham), and then exposed to film (for details, see the online supplement, available at http://atvb.ahajournals.org).

NO Analysis of Cardiac Tissue

Tissue nitroso compounds were quantified using group-specific reductive denitrosation by iodine-iodide with subsequent detection of the NO liberated by gas-phase chemiluminescence as described previously.28 NO-heme was determined by parallel injection of the NO liberated by gas-phase chemiluminescence as described previously.17,28 Briefly, a median sternotomy was performed, and the left coronary artery (LCA) was visualized and ligated proximally using a 7-0 silk suture mounted on a BV-1–tapered needle. The LCA was completely occluded for 30 minutes or 45 minutes, and reperfusion (24 or 72 hours) was initiated by removal of the 7-0 suture (see online supplement).

Myocardial Infarct Size Determination

All of the procedures for area at risk (AAR) and infarct size determination have been previously described (see online supplement).

Echocardiographic Analysis

Transthoracic echocardiography of the LV using a 15-MHz linear array transducer (15L8) interfaced with a Sequoia C25 (Acuson) was performed in mice after 45 minutes of LCA ischemia and 72 hour of reperfusion. Two-dimensional echocardiography was performed as previously described.24 All data were collected from 10 cardiac cycles/experiment.

Statistical Analysis

Data were analyzed where appropriate by Student t test, 1-way ANOVA, or 2-way ANOVA with post hoc Tukey analysis using JMP IN statistical software (SAS Institute). Data are reported as mean±SEM. P values <.05 were considered significant.

Results

Myocardial eNOS Protein Expression in CS eNOS-Tg Mice

Western blot analysis of myocardial protein expression revealed increased eNOS expression, as depicted by the typical 140-kDa band, in the SYS eNOS-Tg mouse as well as robust immunoreactivity in the CS eNOS-Tg mouse (Figure 1A). WT mice displayed low basal levels of myocardial eNOS.

NO Analysis of Cardiac Tissue

WT, SYS-eNOS Tg, and CS-eNOS Tg mouse hearts were analyzed for cardiac tissue NO determinants. Cardiac nitroso levels (Figure 1B) were significantly increased in CS eNOS-Tg mice (279.86±341.00 nmol/L; P<0.001) compared with WT (13.17±3.31 nmol/L) and SYS eNOS-Tg (66.13±9.58 nmol/L) mice. Likewise, Heme-NO adducts (Figure 1C) were also significantly increased (P<0.01) in the CS eNOS-Tg mice (1785.28±256.48 nmol/L) compared with WT (55.96±12.41) and SYS eNOS-Tg (70.88±15.83) mice.

Hemodynamic Analysis of eNOS-Tg Mice

WT, SYS eNOS-Tg, and CS eNOS-Tg mice were implanted with radiotelemetry pressure transducers to assess arterial blood pressure and heart rate (Table). eNOS-Tg (CS and SYS) mice displayed no significant differences in mean arterial blood pressure, systolic blood pressure, or diastolic blood pressure compared with WT controls.

Cardiomyocyte-Specific Overexpression of eNOS Significantly Reduces MI-R Injury

WT, SYS eNOS-Tg, and CS eNOS-Tg mice were first subjected to 30 minutes of LCA ischemia and 24 hours of reperfusion. Representative photomicrographs (see online supplement) from WT, SYS eNOS-Tg, and CS eNOS-Tg hearts demonstrate the protection yielded by eNOS overex-
expression. Quantitatively (Figure 2A), WT mice displayed a mean infarct per AAR (Inf/AAR) of 49.3 ± 2.8% after 30 minutes of myocardial ischemia and 24 hours of reperfusion. SYS eNOS-Tg mice had a 40.9% reduction in Inf/AAR compared with WT mice (29.1 ± 4%; P < 0.01). CS eNOS-Tg mice exhibited a very substantial 63.8% reduction in Inf/AAR compared with WT mice (17.8 ± 2.6%; P < 0.01). This also represented an additional 23% reduction in Inf/AAR compared with SYS eNOS-Tg mice (P < 0.05). In correlation, Inf/LV was also significantly reduced in SYS eNOS-Tg and CS eNOS-Tg mice compared with WT nontransgenic littermates (P < 0.01). Percentage AAR per LV was similar among all groups.

Cardiomyocyte-Specific Overexpression of eNOS Reduces Infarct Size in a More Severe Model of Ischemia-Reperfusion Injury

After 45 minutes of LCA ischemia and 72 hours of reperfusion, mice were assessed for MI (Figure 2B). WT mice exhibited a 56.5 ± 4.1% Inf/AAR. SYS eNOS-Tg mice displayed a mean Inf/AAR of 23.5 ± 6.0% (P < 0.01 versus WT), not significantly different from CS eNOS-Tg mice, which displayed a mean Inf/AAR of 29.2 ± 3.4% (P < 0.01 versus WT). Calculated measurements of Inf/LV correlated well with Inf/AAR. WT mice displayed a mean Inf/LV of 32.1 ± 2.9%. Inf/LV was reduced by 62% in SYS eNOS-Tg and 54% in CS eNOS-Tg mice (P < 0.01 versus WT). There was no difference in AAR/LV between any of the groups.

Cardiomyocyte-Specific Overexpression of eNOS Improves Postischemic Cardiac Function

Echocardiographic analyses of mice at baseline and after 45 minutes of LCA ischemia and 72 hours of reperfusion are reported in Figure 3. Although there was a trend for left ventricular end-diastolic dimensions (LVEDDs) to increase after MI, none of the groups were significantly different (Figure 3A). Mean LVEDD (mm) for WT, SYS eNOS-Tg, and CS eNOS-Tg mice before MI were (3.14 ± 0.07, 3.19 ± 0.09, 2.91 ± 0.06; P = NS). After MI, LVEDD slightly

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<th>Strain</th>
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<th>MABP (mm Hg)</th>
<th>Systolic (mm Hg)</th>
<th>Diastolic (mm Hg)</th>
<th>Heart Rate (bpm)</th>
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<td>4</td>
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<td>120.52 ± 4.79</td>
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<tr>
<td>SYS eNOS-Tg</td>
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<td>97.43 ± 3.48</td>
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<tr>
<td>CS eNOS-Tg</td>
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<td>101.98 ± 2.24</td>
<td>118.48 ± 2.03</td>
<td>84.67 ± 2.88</td>
<td>601.63 ± 4.45†</td>
</tr>
</tbody>
</table>

Mean arterial blood pressure (MABP), systolic blood pressure, diastolic blood pressure, and heart rate were measured in WT, SYS eNOS-Tg, and CS eNOS-Tg mice. Blood pressures were recorded in conscious animals using radiotelemeters with pressure transducers implanted in the aorta.

*P < 0.05 vs WT; †P < 0.05 vs SYS eNOS-Tg.
Measurements of LV wall thickness were not significantly different between any of the groups before or after MI (supplemental Table II, available online at http://atvb.ahajournals.org). Additionally, heart to body weight ratios were calculated for all groups after infarction. There was no significant difference between any of the groups (data not shown).

**Discussion**

It is now generally well accepted that NO therapy is remarkably beneficial in the setting of MI-R injury. We reported previously that systemic genetic overexpression of eNOS is protective in animal models of both MI-R injury and congestive heart failure. These studies revealed that the protection afforded by eNOS overexpression was NO dependent. Given the current interest in translating gene therapy to the clinic, we sought to further clarify the protective effects of tissue-specific eNOS overexpression versus global overexpression. Using 2 distinct transgenic mice, we compared systemic versus tissue-specific (cardiomyocyte-specific) eNOS overexpression. Both mice used the same human transgene, but each was under the control of different promoters. The SYS eNOS-Tg mouse used the native eNOS promoter, as well as an endothelium enhancer element, thereby promoting systemic transgene expression that was restricted primarily to the vascular endothelium. Site-specific overexpression was achieved in the CS eNOS-Tg mouse by using the α-MHC promoter, thereby restricting overexpression to cardiomyocytes. Both of these mice have previously been well characterized. Both the SYS eNOS-Tg and CS eNOS-Tg mice displayed increased cardiac eNOS protein expression. However, eNOS protein expression in the CS-eNOS mouse was found to be more robust. This expression is limited to cardiomyocytes and has been shown previously to display normal cellular localization, including association with caveolin-3.

The abundant increase in protein expression in the CS-eNOS mouse translated into very high NO production, as is evident by measurements of cardiac tissue nitroso levels and cardiac heme-NO adduct levels. Although not significant, the SYS eNOS-Tg mouse also displayed higher levels of cardiac...
tissue NO determinants than those seen in the WT mouse. These data provide conclusive evidence that genetic overexpression in the CS eNOS-Tg mouse not only translated into increased protein expression but consequently increased NO production. These results also allow for the possibility that any increased protection seen in the CS eNOS-Tg mouse may relate solely to increased levels of NO. However, it has been previously suggested that such high levels of NO would only seem to exaggerate or increase ischemic injury in these mice. Reports have shown that high levels of NO, such as those seen in cases of iNOS overexpression, may be deleterious. However, in the current study, we found the high tissue levels of NO measured in the CS eNOS-Tg mouse were not injurious but quite protective in the setting of MI-R injury. This finding leaves open the possibility that targeted therapy, as in the CS eNOS-Tg mouse, allows for a much larger therapeutic dose window than would high levels of globally generated NO. One explanation for this is that the high levels of myoglobin in the heart have been suggested to buffer excess NO and thereby ablate any pathogenic occurrences resulting from extreme NO production. Suggestive of this theory is the high levels of heme-NO adduct observed in the CS eNOS-Tg mouse.

Arterial blood pressure analysis of WT and eNOS-Tg mice revealed no statistical differences in mean arterial blood pressure, diastolic, or systolic blood pressure. These results suggest that the hemodynamic status of all mice were very similar at baseline before MI-R. This result is somewhat surprising given the high levels of NO found in the CS-eNOS-Tg mouse and indicates that these mice have either become less sensitized to NO-mediated vasodilation or have developed compensatory mechanisms that prevent any alterations in hemodynamic status.

After 30 minutes of LCA ischemia and 24 hours of reperfusion, we found cardiac-specific (CS) eNOS overexpression to profoundly limit myocardial infarct size. CS eNOS-Tg mice had a 64% reduction in Inf/AAR compared with WT mice. SYS eNOS-Tg mice displayed a 41% reduction in Inf/AAR compared with WT mice. These results indicate that myocyte-specific overexpression was clearly more advantageous, in this model, reducing infarct size an additional 23% beyond that of systemic overexpression. However, in a more injurious model in which mice were subjected to 45 minutes of ischemia and 72 hours of reperfusion, CS eNOS-Tg mice had similar injury as SYS
eNOS-Tg mice. Both mice yielded significant cardioprotection compared with WT mice but were not significantly different from one another. These results indicate that CS overexpression is more advantageous in limiting infarct size but that increasing the magnitude of myocardial injury may ablate any protection beyond that of systemic overexpression.

Echocardiographic analysis of LV FS revealed no significant differences between all groups after MI. There was a trend for CS eNOS-Tg mice to have a significantly higher percent fractional shortening, but this observation was present at both baseline and post MI. Further examination of this trend revealed that CS eNOS-Tg mice displayed no significant increase in LVEF after infarction, whereas SYS eNOS-Tg and WT mice both displayed significant increases in LVEF. These data suggest that CS eNOS-Tg mice had less systolic dysfunction after infarction. However, these conclusions are slightly clouded by the fact that CS eNOS-Tg mice did display slightly decreased LVEDV at baseline. Further examination of cardiac function after MI revealed that CS eNOS-Tg mice had no significant decrease in ejection fraction after LCA occlusion. WT mice and SYS eNOS-Tg mice both had a significant (P<0.05) ~30% decrease in ejection fraction, whereas CS eNOS-Tg had only a nonsignificant 14% reduction after infarction. All together, these data suggest that although the significant decrease in infarct size was lost after 45 minutes of LCA ischemia and 72 hours of reperfusion, there was a trend for CS eNOS-Tg mice to have greater preservation of LV function following ischemic injury.

A number of mechanisms have been suggested to contribute to the cardioprotection afforded by eNOS overexpression. eNOS overexpression has been attributed to increased negative modulation of adrenergic-mediated increases in inotropy, thereby acting as an endogenous β-blocker in the ischemic or failing myocardium. Protection yielded via transgenic overexpression of eNOS could also be credited to the enhanced control of myocardial oxygen consumption. Additional reports have suggested that eNOS-mediated increase in cGMP downregulates adhesion molecule expression and subsequent leukocyte recruitment. In conclusion, increased eNOS expression has been shown to be a powerful agent in preconditioning against future ischemic episodes.

In conclusion, these results provide evidence that site-specific targeting of gene therapy may be more advantageous in limiting MI-R injury and subsequent cardiac dysfunction. We have shown that cardiomyocyte-specific eNOS overexpression resulting in increased cardiac NO significantly decreases infarct size beyond that of systemic overexpression. Additionally, CS eNOS-Tg mice tended to have better preservation of cardiac function compared with SYS eNOS-Tg mice after MI. More research is needed to better understand the protective pathways involved in CS eNOS–transgenic-mediated cardioprotection.

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Disclosures
None.

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Supplemental Methods.

**Western Blot Analysis of eNOS.** Myocardial tissue lysates were centrifuged to remove any particulate, and protein concentration of the cleared lysate was measured using the BioRad Dc protein assay. Equal amounts of protein (40 µg) were loaded into each lane and separated on a 7% polyacrilamide gel. Protein was transferred to PVDF overnight at 30 V and then blocked in 5% milk in tris-buffered saline tween-20 (TBST) at RT for 3 hr. Membranes were washed 3 times with TBST and then incubated with mouse-anti-eNOS (1:4000, BD Transduction Labs), in 5% BSA TBST overnight at 4°C. Membranes were then washed 3 times with TBST and then incubated with HRP-linked anti-mouse secondary (Amersham) at 1:2000 in 5% BSA TBST at room temperature for 3 hr. Membranes were then washed 3 times with TBST, incubated with ECL reagents (Amersham), and then exposed to film. Membranes used to detect eNOS were then stripped and incubated with mouse-anti-β-actin (as a loading control) at 1:2000 in 5% BSA TBST at 4°C overnight. Membranes were then washed and incubated with HRP linked anti-mouse secondary at 1:2000 in 5% BSA TBST at room temperature for 3 hr., washed and incubated with ECL reagents, and then exposed to film. Densitometric analysis was performed using Image J software from the NIH.

**Nitric Oxide Analysis of Cardiac Tissue.** Hearts were flushed completely free of blood by ex vivo perfusion with air-equilibrated PBS supplemented with 10mM N-ethylmaleimide (NEM, Pierce #23030) and 2.5mM EDTA (Fisher #BP120). Tissue nitroso compounds were quantified using group-specific reductive
denitrosation by iodine-iodide with subsequent detection of the NO· liberated by gas-phase chemiluminescence as previously described {Feelisch, 2002}. NO·-heme was determined by parallel injection of replicate aliquots of tissue homogenates into a solution of 0.05M ferricyanide in PBS at pH 7.5 and 37°C. This method employs one-electron oxidation rather than reduction to achieve denitrosation, with the liberated NO· being quantified by gas-phase chemiluminescence and has been previously described in detail {Bryan, 2004}.

**Myocardial Ischemia-Reperfusion Protocol.** Surgical procedures used in the MI-R protocol were similar to methods previously described {Jones, 1999}{Jones, 2001}. Briefly, mice were weighed and anesthetized via intraperitoneal injections of pentobarbital sodium (50 mg/kg) and ketamine (60 mg/kg). Mice were then orally intubated and connected to a Harvard Apparatus Rodent Ventilator (Model 835) with 100% oxygen supplementation (225 µl stroke volume, 115 strokes/min). Body temperature was monitored using a rectal probe thermometer and controlled with an infrared heat lamp. A median sternotomy was performed and the left coronary artery (LCA) was visualized and ligated proximally using a 7-0 silk suture mounted on a BV-1 tapered needle. A piece of PE-10 tubing was placed between the left coronary artery and the 7-0 silk suture to minimize coronary artery trauma induced by occlusion and to facilitate reperfusion. The LCA was completely occluded for 30 min. or 45 min. and reperfusion (24 or 72 hr.) was initiated by removal of the 7-0 suture. Following reperfusion the chest wound was reapproximated and closed using 6-0 BIOSYN suture. Then mice were extubated and allowed to recover with supplemental
oxygen until mobile. All mice received butorphanol tartrate (0.3 mg/kg, sc) as needed to minimize pain.

**Myocardial Infarct Size Determination.** After 24 or 72 hr. of reperfusion, the mice were anesthetized as described previously, intubated, and connected to a rodent ventilator. A catheter (PE-10 tubing) was placed in the common carotid artery to allow for Evans blue dye injection. A median sternotomy was performed, and the LCA was religated in the same location as before. Evans blue dye (1 mL of a 3% solution) was injected into the carotid artery catheter to delineate the ischemic zone from the nonischemic zone. The heart was rapidly excised and cross-sectioned into 1-mm-thick sections, which were then incubated in 1.0% 2,3,5-triphenyltetrazolium chloride (dissolved in normal saline) for 5 min at 37°C to demarcate the viable and nonviable myocardium within the risk zone. Each of the five 1-mm-thick myocardial slices was weighed, and the areas of infarction, risk, and nonischemic LV were assessed by a blinded observer using computer-assisted planimetry (NIH Image 1.57). All of the procedures for area at risk (AAR) and infarct size determination have been previously described {Jones, 1999}{Jones, 2001}.

**Hemodynamic Measurements.** Aortic blood pressures (systolic, diastolic, and mean) and heart rate were measured in wild type, SYS-eNOS Tg, and CS-eNOS Tg mice in the conscious state using radiotelemetry techniques. Mice were surgically implanted with the PA-C10 radiotelemeter pressure transducers (DSI, St. Paul, MN) and the catheters were placed in the aorta as previously described {Whitesall, 2004}. Mice were anesthetized with ketamine (50 mg/kg, ip) and
xylazine (8mg/kg, i.p.) and the skin on the ventral surface was treated with chemical hair remover. The skin was then washed, wiped clean with topical antiseptic and then wiped with alcohol. An incision was made from the chin to the superior sternum. The left carotid artery was surgically exposed from the bifurcation of internal and external carotid toward the heart. About 1 cm of the common carotid artery was isolated and ligated at the bifurcation using 4-0 silk suture and an additional tie was placed midway on the vessel for securing the catheter and hemostasis. Distal and proximal traction was used to stabilize the vessel. A small incision was made in the artery adjacent to the bifurcation and the catheter was placed in the artery and advanced 1.2 cm so that the tip of the catheter was in the aorta. A pulsing radio signal was used to confirm a functional implant by a distinctive high-frequency oscillating tone on a portable AM radio set at 500 kHz. The catheter was then firmly tied in place and the radiotelemeter was secured in place under the skin of the right flank with tissue adhesive. All skin wounds were closed with interrupted 6-0 silk sutures. The mice were treated with antibiotic (Cefazolin, GlaxoSmithKline, 80 mg/kg, sc). In addition, the mice also received buprenorphine (Buprenex, 0.3 mg/kg) for analgesia. Mice were placed in a warm recovery area supplemented with 100% oxygen and allowed to recover. At 7 days following implantation hemodynamic measurements were conducted using DSI Open Art interfaced with IOX software (EMKA Technologies, Church Falls, VA). Measurements were conducted 6 times each 24-hour cycle over a 48-hour sampling period.
**Supplemental Figure I.** Representative photomicrographs of hearts from wild-type mice, mice with systemic eNOS overexpression (SYS eNOS-Tg), and mice with cardiac specific eNOS overexpression (CS eNOS-Tg). Photographs were taken following 30 min. of myocardial ischemia and 24 hours of reperfusion. Hearts were stained with Evans blue dye *in vivo* followed by staining in 2,3,5-triphehyltetrazolium chloride (TTC). Blue staining represent nonischemic zones, red areas demarcate ischemic-reperfused viable myocardium, and pale staining areas represent necrotic cardiac myocytes within the ischemic-reperfused tissue (infarct area). Myocardial infarct size was significantly attenuated in both the SYS eNOS-Tg and CS eNOS-Tg hearts compared to the nontransgenic, wild-type control heart.
Table I. Cardiac Performance Pre- and Post-Myocardial Infarction.
Wild-type, systemic eNOS-Transgenic (SYS eNOS-Tg), and cardiomyocyte specific eNOS-Transgenic (CS eNOS-Tg) mice were echocardiographically assessed at baseline (BASE) and post 45 min of left coronary ischemia and 72 hr of reperfusion. (HR=heart rate, SV=stroke volume, CO=cardiac output, Vmax=(maximum aortic velocity) [* = p < 0.05 vs. baseline, † = p < 0.05 vs. Wild-Type post analysis]

<table>
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<tr>
<th>Strain</th>
<th>n</th>
<th>HR (bpm)</th>
<th>SV (µL)</th>
<th>CO (mL/min)</th>
<th>Vmax (mm/sec)</th>
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Table II. Echocardiograph dimensions Pre- and Post-Myocardial Infarction.
Wild-type, systemic eNOS-Transgenic (SYS eNOS-Tg), and cardiomyocyte specific eNOS-Transgenic (CS eNOS-Tg) mice were echocardiographically assessed at baseline (BASE) and post 45 min of left coronary ischemia and 72 hr of reperfusion. (IVS = interventricular septum, PW = LV posterior wall, d = diastole, s = systole)

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<th>PW-d (mm)</th>
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<td>1.300 ± .053</td>
<td>1.121 ± .060</td>
<td>1.462 ± .056</td>
</tr>
<tr>
<td>CS eNOS-Tg</td>
<td>16</td>
<td>0.875 ± .032</td>
<td>1.387 ± .039</td>
<td>0.994 ± .050</td>
<td>1.356 ± .053</td>
</tr>
<tr>
<td>Wild-Type</td>
<td>18</td>
<td>0.951 ± .045</td>
<td>1.250 ± .048</td>
<td>0.939 ± .024</td>
<td>1.311 ± .043</td>
</tr>
<tr>
<td>SYS eNOS-Tg</td>
<td>10</td>
<td>0.950 ± .043</td>
<td>1.230 ± .073</td>
<td>0.980 ± .055</td>
<td>1.350 ± .065</td>
</tr>
<tr>
<td>CS eNOS-Tg</td>
<td>11</td>
<td>1.000 ± .040</td>
<td>1.355 ± .080</td>
<td>1.082 ± .050</td>
<td>1.525 ± .043</td>
</tr>
</tbody>
</table>