Inducible nitric oxide synthase inhibits oxygen consumption in collateral-dependent myocardium

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Chen Y, Zhang P, Li J, Xu X, Bache RJ. Inducible nitric oxide synthase inhibits oxygen consumption in collateral-dependent myocardium. Am J Physiol Heart Circ Physiol 306: H356–H362, 2014. First published December 6, 2013; doi:10.1152/ajpheart.00308.2013.—Following coronary artery occlusion growth of collateral vessels can provide an effective blood supply to the dependent myocardium. The ischemia, which results in growth of collateral vessels, recruits an inflammatory response with expression of cytokines and growth factors, upregulation of endothelial nitric oxide (NO) synthase (eNOS) in vascular endothelial cells, and expression of inducible nitric oxide synthase (iNOS) in both vessels and cardiac myocytes. Because NO is a potent collateral vessel dilator, this study examined whether NO derived from iNOS or constitutive NOS regulates myocardial blood flow (MBF) in the collateral region. Nonselective NOS inhibition with Nω-nitro-l-arginine (LNA) caused vasoconstriction with a significant decrease in MBF to the collateral region during exercise. In contrast, the highly selective iNOS inhibitor 1400W with nonselective NOS blockade was proportionate to an increase in myocardial O2 consumption (MV˙O2). The results suggest that NO produced by iNOS inhibits MV˙O2 in the collateralized region, so that the increase in MBF following iNOS blockade was the result of metabolic vasodilation secondary to an increase in MV˙O2. Thus the coordinated expression of iNOS to restrain MV˙O2 and eNOS to maintain collateral vasodilation act to optimize the O2 supply-demand relationship and protect the collateralized myocardium from ischemia.

NITRIC OXIDE (NO) produced by constitutively expressed endothelial NO synthase (eNOS) is known to mediate the vasodilator responses to endothelium-dependent agonists such as acetylcholine and bradykinin, and is responsible for the flow-mediated coronary artery dilation that occurs in response to increased endothelial shear stress (27). In addition to effects on vessels, myocardial ischemia or hypoxia has been found to cause increased expression of iNOS in cardiac myocytes (20) where an increase in NO production might cause inhibition of O2 consumption. Thus an increase of either eNOS or iNOS expression in the collateral vessels could act to maintain vasodilation and augment blood flow into the collateralized region, whereas iNOS expressed in the cardiac myocytes might influence mitochondrial respiration. The present study was performed to determine whether the NO that causes tonic dilatation of the coronary collateral vessels is produced by eNOS or by iNOS. We compared the effects of the highly selective iNOS inhibitor 1400W with nonselective NOS blockade with LNA on blood flow in a collateral-dependent region of myocardium of dogs with coronary artery occlusion (13). We also measured oxygen consumption to determine whether NO generated by either eNOS or iNOS alters MV˙O2 in the collateral-dependent region.

METHODS

Surgical instrumentation. Studies were performed in 15 adult mongrel dogs (25–35 kg) in accordance with the “Position of the American Heart Association on Research Animal Use” and approved by the Animal Care Committee of the University of Minnesota. The animals were anesthetized with pentobarbital sodium (30–35 mg/kg iv), intubated, and ventilated with 1.5% isoflurane. A left thoracotomy was performed, and heparin-filled polyvinyl chloride catheters (3.0 mm OD) were introduced into the ascending aorta, left atrium, and left ventricle (LV). A similar catheter was introduced into the right atrium and advanced through the coronary sinus to the origin of the anterior interventricular vein to permit venous blood sampling from the region perfused by the left anterior descending coronary artery (LAD). The proximal LAD was fitted with a Doppler velocity probe (Craig Hartley, Houston, TX) and hydraulic occluder.

Induction of collateral vessel growth. Collateral vessel development was produced using a modification of the Franklin intermittent coronary artery occlusion protocol (12). Beginning 10 to 14 days postoperatively, eight 2-min coronary occlusions were performed every 15 min for 2 h, 5 days each week by inflating the hydraulic occluder while the Doppler signal was observed to insure total coronary occlusion. After occlusions had been performed for 3 wk, the artery was permanently occluded. We previously observed that

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this degree of collateralization is sufficient to allow permanent coronary occlusion without producing infarct (31). Studies were performed after permanent occlusion had been in place for 3 days.

Myocardial blood flow measurements. Myocardial blood flow (MBF) was measured using left atrial injection of 2 × 10^6 15 μm diameter microspheres labeled either with radionuclides (NEN, Boston, MA) or with fluorescent dyes (Triton Dyes, San Diego, CA).

Experimental protocol. Heart rate and arterial and LV pressures were measured with fluid-filled catheter, and microspheres were injected for determination of MBF during quiet resting conditions. Dogs were then exercised on a treadmill at 6.4 km/h with a 5–10% grade to achieve heart rates ~200 beats/min. All measurements were repeated and microspheres injected when steady-state exercise had been present for at least 3 min. After completion of these control measurements, animals were rested for 30 min and the selective iNOS inhibitor 1400W was infused in a dose of 1.5 mg/kg iv over 10 min in eight dogs. Thirty minutes after the 1400W infusion, all rest and exercise measurements were obtained as previously described. To insure that adequate iNOS inhibition had been achieved, in three animals a second infusion of 1400W, 10 mg/kg, was administered and rest and exercise measurements were repeated.

In eight animals (3 of which were also studied with 1400W), the effect of nonselective NOS blockade with LNA was studied. After completion of control rest and exercise measurements, LNA was infused in a dose of 20 mg/kg iv over 10 min. This dose of LNA was previously found to cause 80% inhibition of the endothelium-dependent vasodilation elicited by acetylcholine infused into the LAD of awake dogs (1). One hour after the LNA infusion, all rest and exercise measurements were repeated.

Determination of MBF. Hearts were fixed in 10% formalin and duplicate myocardial specimens removed from the collateral-dependent anterior LV wall and from the region perfused by the left circumflex artery to represent remote normally perfused myocardium. Specimens were divided into three transmural layers from endocardium to epicardium for measurement of the transmural distribution of MBF. Coronary conductance per gram of myocardium was calculated as MBF/mean aortic pressure.

Myocardial oxygen consumption. Aortic and coronary venous PO2, PCO2, and pH were determined with a blood gas analyzer (Instrumentation Laboratory Model 113). Blood oxygen content was calculated as 

\[
\text{MBF} = \frac{(0.0136 \times \text{hemoglobin} \times \text{percent oxygen saturation}) + (P_{O2} \times 0.0031)}{Hb} 
\]

MVO2 in the LAD distribution was calculated as the arteriovenous difference of oxygen content multiplied by mean MBF.

Western blot. LV myocardium was homogenized in lysis buffer containing 20 mM Tris-HCl, 150 mM NaCl, 1 mm EGTA, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na2VO4, 1 μM leupeptin, 2 μg/ml aprotinin, and 1 mM PMSF. The homogenates were clarified by centrifugation at 12,000 g at 4°C for 10 min, and equal amounts of protein (80 μg) were subjected to 10% SDS-polyacrylamide gel, transferred to Hybond nitrocellulose membrane, incubated with primary antibody followed by horseradish peroxidase-labeled secondary antibody, and detected by enhanced chemiluminescent substrate (Amersham). iNOS antibody was from Santa Cruz (SC-651) and eNOS mouse antibody from BD Transduction Laboratories (610297). Signal intensities were quantified using ImageJ and are presented as the arbitrary ratio to the signal intensities of sarcomeric α-actin.

NOS activity assay. NOS activity was measured using a NOS activity Assay kit (Cayman Chemical 781001) measuring the conversion of L-[3H]arginine to L-[3H]citrulline in the presence of saturating concentrations of the cofactors of the enzyme with or without calcium.

Data analysis. Data are expressed as means ± SE. Statistical analysis was performed using two-way ANOVA with P < 0.05 regarded as significant. When a significant result was found, comparisons between groups were made with the Student’s t-test with the Bonferroni correction. Comparisons between normal and collateral zones were performed using the independent t-test (two-tailed) with Bonferroni correction.

RESULTS

Selective iNOS blockade. Hemodynamic data at rest and during exercise in eight collateral dogs are shown in Table 1. Heart rate increased from 123 ± 5 beats/min at rest to 181 ± 8 during exercise. Mean aortic pressure, LV systolic pressure, and the rate pressure product increased significantly during exercise. Selective iNOS blockade with 1400W caused no significant change in any of these hemodynamic variables either at rest or during exercise. Mean MBF is shown in Table 2. In the remote zone mean MBF

Table 1. Hemodynamic measurements in dogs with a collateral-dependent myocardial region during control conditions and after selective iNOS inhibition with 1400W

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1400W</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>123 ± 5.3</td>
<td>120 ± 7.2</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>127 ± 1.6</td>
<td>126 ± 2.5</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>100 ± 1.8</td>
<td>101 ± 1.5</td>
</tr>
<tr>
<td>Rate pressure product, mmHg·beats/min</td>
<td>16.5 ± 0.9</td>
<td>15.7 ± 0.8</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>127 ± 1.6</td>
<td>126 ± 2.5</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>100 ± 1.8</td>
<td>101 ± 1.5</td>
</tr>
<tr>
<td>Rate pressure product, mmHg·beats/min</td>
<td>16.5 ± 0.9</td>
<td>15.7 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 to 8. *P < 0.05 comparing rest vs. exercise.

Table 2. Myocardial blood flow, coronary venous oxygen tension, and myocardial oxygen consumption in dogs with a collateral-dependent region during control conditions and after selective iNOS inhibition with 1400W

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1400W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone myocardial blood flow, ml·min⁻¹·g⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remote</td>
<td>1.21 ± 0.14</td>
<td>1.12 ± 0.11</td>
</tr>
<tr>
<td>Collateral</td>
<td>0.96 ± 0.15</td>
<td>0.95 ± 0.12</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>13.5 ± 0.6</td>
<td>13.5 ± 0.6</td>
</tr>
<tr>
<td>Coronary venous PO2, torr</td>
<td>16.5 ± 1.1</td>
<td>17.0 ± 1.5</td>
</tr>
<tr>
<td>Collateral zone MVO2, ml·min⁻¹·g⁻¹</td>
<td>0.112 ± 0.013</td>
<td>0.123 ± 0.016</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 to 8. *P < 0.05 comparing rest vs. exercise; †P < 0.05 comparing control vs. 1400W. PO2, oxygen tension.
was 1.21 ± 0.14 during resting conditions and increased significantly in response to exercise (P < 0.001). In the collateral zone MBF at rest was less than in the remote zone owing to significantly lower flow in the subendocardium with no significance difference in midwall or subepicardial flow. Exercise caused an increase of mean MBF in the remote zone to 2.11 ± 0.25 vs. 1.22 ± 0.13 in the collateral zone (P < 0.001). Coronary conductance for three transmural layers from subendocardium to subepicardium is shown in Figs. 1 and 2. Conductance increased significantly in all transmural layers of the remote zone during exercise. In contrast, in the collateral zone coronary conductance increased significantly only in the subepicardial layer in response to exercise, with no significant increases in the midwall or subendocardial layers.

As a result, the subendocardial/subepicardial (endo/epi) blood flow ratio in the collateral zone decreased from 1.13 ± 0.18 at rest to 0.7 ± 0.17 during exercise (P < 0.05). The endo/epi blood flow ratio in the remote zone was 1.38 ± 0.6 at rest and tended to decrease during exercise, although this was not significant (1.22 ± 0.04; P = ns).

As shown in Table 2, MV¯O₂ in the collateral zone increased in response to exercise as the result of an increase in MBF as well as a decrease in PO₂ in the anterior interventricular vein draining the collateralized region (P < 0.05 in comparison with rest). 1400W had no effect on MBF or coronary conductance in either the collateral zone or the remote zone during resting conditions and had no effect on coronary venous PO₂ or MV¯O₂. However, during exercise 1400W caused a 21% mean increase in MBF in the collateral zone from 1.22 ± 0.13 to 1.47 ± 0.15 ml·min⁻¹·g⁻¹ (P < 0.05) (Table 2). This resulted from a significant increase in mean collateral zone conductance from 0.0131 ± 0.0013 during control exercise to 0.0167 ± 0.0018 during exercise after 1400W (P < 0.05) with change in normal zone conductance (0.0226 ± 0.0023 to 0.0238 ± 0.0015 ml·mmHg·min⁻¹·g⁻¹). The increase in exercise MBF in the collateral zone caused by 1400W was essentially uniform across all transmural layers with no change in the transmural distribution of flow or conductance (Fig. 1). As a result of the increase in MBF, as well as a trend toward a decrease of coronary venous PO₂, 1400W caused a 26% increase in MV¯O₂ in the collateral-dependent myocardium during exercise (P < 0.05). As shown in Fig. 3, 1400W did not change the relationship between MBF and MV¯O₂.

Nonselective NOS blockade. LNA caused significant increases of mean aortic pressure and LV systolic pressure, whereas heart rate decreased significantly (Table 3). Because of the opposite effects on heart rate and systolic pressure, there was no change in rate-pressure product. LNA caused no change in MBF during resting conditions. However, during exercise LNA caused coronary vasoconstriction with significant decreases of coronary conductance in both the collateral and remote zones (Fig. 2) with a mean 26 ± 5% decrease of blood flow in the collateral zone as compared with control

![Fig. 1. Coronary conductance in 8 animals at rest and with treadmill exercise during control conditions and after selective inducible nitric oxide synthase (iNOS) inhibition with 1400W. Conductance in the collateral zone and the remote zone is shown for 3 transmural layers across the left ventricular wall from epicardium (Epi) to endocardium (Endo). *P < 0.05 comparing control vs. 1400W. Mid, midwall.]

![Fig. 2. Coronary conductance in 6 animals at rest and with treadmill exercise during control conditions and after nonselective NOS inhibition with N^G-nitro-L-arginine (LNA). Conductance in the collateral zone and the remote zone is shown for 3 transmural layers across the left ventricular wall from epicardium (Epi) to endocardium (Endo). *P < 0.05 comparing control vs. LNA. Mid, midwall.]
exercise (Table 3). The decreases in coronary conductance caused by LNA were similar in all three layers of the LV wall in both the remote and collateral zones (Fig. 2). LNA caused a significant decrease of $\text{MV} \dot{\Omega}_2$ in the collateral zone during exercise that resulted from a decrease of MBF as well as a nonsignificant trend toward a decrease in coronary venous $P \Omega_2$ (Table 3).

**NOS protein and activity.** Western blots of myocardial specimens from four normal dogs and five dogs exposed to repetitive coronary occlusions are shown in Fig. 4. iNOS protein was nearly undetectable in normal hearts but was markedly increased in collateral zone myocardium. iNOS was also increased to a lesser extent in the remote zone of the collateralized hearts. eNOS protein was not significantly different from normal in either the collateral zone or the remote zone of the collateralized hearts. In agreement with the increase in iNOS, calcium-independent NOS activity was increased 250% in the collateral zone with a lesser but still significant increase in the remote zone (Fig. 5). There was no significant difference in calcium-dependent NOS activity between normal hearts and either the collateral or remote zones of the collateralized hearts.

**DISCUSSION**

We previously reported that nonselective NOS inhibition with LNA caused a decrease in blood flow to collateral-dependent myocardium in dogs during exercise, indicating that NO contributes to maintaining vasodilation of vessels that supply the collateral zone (22, 31). Because previous reports have demonstrated that iNOS expression is increased in developing collateral vessels (4, 26, 30), this study was performed to determine whether vasodilation of the collateral vessels is dependent on NO produced by iNOS. The results were contrary to our expectation; rather than causing a decrease of collateral flow, selective iNOS inhibition caused a significant increase of MBF in the collateral region during exercise. The increase in collateral zone blood flow following selective iNOS blockade was proportionate to the increase in oxygen consumption of the collateralized myocardium. This suggests that the principal effect of NO produced by iNOS in the collateralized region was to reduce oxygen consumption and that the increase in coronary flow following iNOS blockade was the result of metabolic vasodilation of the resistance vessels in the collateral zone in response to the increase in $\text{MV} \dot{\Omega}_2$. The possible mechanisms and implications of these changes caused by blockade of NO synthesis are discussed below.

Table 3. **Hemodynamic measurements and myocardial blood flow in dogs with a collateral-dependent myocardial region during control conditions and after nonselective NOS inhibition with LNA**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rest</th>
<th>LNA</th>
<th>Exercise</th>
<th>LNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>148 ± 8.9</td>
<td>127 ± 12</td>
<td>222 ± 10*</td>
<td>203 ± 11†</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>116 ± 4.7</td>
<td>131 ± 5.9†</td>
<td>133 ± 4.3*</td>
<td>157 ± 9.4†</td>
</tr>
<tr>
<td>Rate pressure product, mmHg/beats/min</td>
<td>17.6 ± 1.4</td>
<td>16.7 ± 1.2</td>
<td>28.8 ± 1.5*</td>
<td>31.2 ± 3.5*</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>99 ± 4.1</td>
<td>124 ± 3.2†</td>
<td>116 ± 3.7*</td>
<td>136 ± 4.6†</td>
</tr>
<tr>
<td>Zone myocardial blood flow, ml·min⁻¹·g⁻¹</td>
<td>Normal: 1.26 ± 0.11</td>
<td>1.17 ± 0.22</td>
<td>2.51 ± 0.16*</td>
<td>1.89 ± 0.07†</td>
</tr>
<tr>
<td></td>
<td>Collateral: 0.92 ± 0.05</td>
<td>0.90 ± 0.35</td>
<td>1.85 ± 0.31*</td>
<td>1.40 ± 0.28‡</td>
</tr>
<tr>
<td></td>
<td>Coronary venous $P \Omega_2$, torr</td>
<td>NA</td>
<td>14.7 ± 0.9</td>
<td>14.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Collateral zone $\text{MV} \dot{\Omega}_2$, ml·min⁻¹·g⁻¹</td>
<td>NA</td>
<td>0.173 ± 0.03</td>
<td>0.095 ± 0.02‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 3$ for $\text{MV} \dot{\Omega}_2$ and $P \Omega_2$ and $n = 5$ to 7 for other parameters. *$P < 0.05$ comparing rest vs. exercise; †$P < 0.05$ comparing control vs. N⁶-nitro-l-arginine (LNA). NA, not available.

Fig. 3. Myocardial blood flow plotted against myocardial oxygen consumption at rest and during exercise for 8 dogs during control conditions and after selective iNOS inhibition with 1400W.

Fig. 4. Western blots for iNOS and endothelial nitric oxide synthase (eNOS) from myocardium of normal canine hearts and from the collateral zone and remote zone of dogs with a collateral-dependent myocardial region. *$P < 0.05$ vs. normal zone myocardium; †$P < 0.05$ vs. collateral zone myocardium.
There are several mechanisms by which NO produced by iNOS could reduce oxygen consumption in the collateralized region. NO has been shown to cause rapid and reversible inhibition of oxygen consumption of vascular endothelial cells (8) as well as in mitochondria isolated from the heart (3, 6). This occurs as NO competes at the oxygen binding site of cytochrome oxidase, thereby causing reversible inhibition of oxygen consumption (3, 8). In the present study 1400W tended to cause an increase in MV$\dot{O}_2$ in the collateral zone during resting conditions, but this increase became significant only during exercise. The sensitivity of mitochondrial respiration to NO is dependent on the oxygen concentration and the respiratory state of the mitochondria, consistent with NO competing with oxygen at cytochrome $c$ oxidase (3, 8). The lower mitochondrial $O_2$ tension during exercise, in conjunction with the higher respiratory rate, could explain the greater effect of NO on oxygen consumption during exercise. In addition to direct effects on mitochondrial respiration, studies in cardiac myocytes (5), as well as in laboratory animals (15) and human subjects with LV dysfunction (16), have demonstrated that NO attenuates the positive inotropic response to $\beta$-adrenergic agonists. It is thus possible that increased NO production by iNOS in the collateral-dependent region could attenuate the inotropic response that occurs during exercise and thereby decrease oxygen requirements. Because adrenergic activity is low during resting conditions, this effect would occur predominantly during exercise. Reactive oxygen or nitrogen species generated from uncoupled NO (including peroxynitrite) can also inhibit respiration by causing protein modifications of several components of the mitochondrial respiratory chain (6); however, these effects are irreversible or only slowly reversible and so could not fully explain the increase in MV$\dot{O}_2$ seen over the relatively short observation period in the present study.

There are several sources of NO in the cardiomyocyte. Both eNOS localized to the coronary endothelium and the sarcolemma of the cardiomyocytes, and neuronal NO synthase (nNOS) colocalized with the sarcoplasmic reticulum, represent sources of NO. There are conflicting data as to whether NO produced by eNOS in the coronary endothelium can influence oxygen consumption of the in vivo heart. In perfused rat hearts, bradykinin or carbachol (both of which activate eNOS in the endothelium) were reported to inhibit MV$\dot{O}_2$ (29). In contrast, Kojic et al. (24) reported that bradykinin administered to perfused mouse hearts caused no change in MV$\dot{O}_2$ despite a fivefold increase in NO production, suggesting that NO from eNOS in the endothelium did not reach the mitochondria. These investigators subsequently demonstrated that MV$\dot{O}_2$ could be inhibited only at intra-arterial concentrations of authentic NO far above those that could be achieved by administration of endothelium-dependent agonists (23). An important consideration in the heart is that oxymyoglobin in myocytes binds NO to produce metmyoglobin and nitrate, thereby limiting NO diffusion within the cell (11, 25). For this reason, in cells containing oxymyoglobin NO tends to be localized to the site of production, explaining how eNOS and nNOS can have differing effects in the heart (2).

Because of the scavenging effect of oxymyoglobin, NO produced near the mitochondria would be more likely to exert effects on respiration than NO generated by eNOS in the sarcolemma or the coronary endothelium. In this context, NO generated by iNOS expressed diffusely in the cytosol might have greater access to the mitochondria than NO produced by constitutive NOS. Furthermore, the ability of iNOS to generate relatively large amounts of NO independent of calcium has the potential both to inhibit mitochondrial respiration and to oxidize myoglobin, thereby hindering oxygen delivery to the mitochondria. It should be mentioned that myocardial mitochondria have been reported to possess NO synthase located on the inner mitochondrial membrane (21). Although not fully characterized, myocardial mitochondrial iNOS has been identified as the $\alpha$-isofrom of nNOS. Because 1400W is only a very weak inhibitor of nNOS, it is unlikely that the observed effects on respiration could be ascribed to nonselective inhibitory effects of 1400W (28).

The present findings suggest that NO has differing effects on normal and collateralized hearts. In contrast with normal hearts where iNOS blockade caused no change in MV$\dot{O}_2$ (7), selective blockade of iNOS caused an increase of MV$\dot{O}_2$ in the collateral zone. Both in the normal heart and in the remote region of the collateralized heart, LNA caused no significant change in MBF during resting conditions. In contrast, in the collateralized hearts nonselective NOS blockade with LNA caused collateral vasoconstriction with a decrease of blood flow in the dependent myocardium. Thus NO has greater importance for maintaining energy balance in the collateralized hearts during exercise, where the coordinated expression of iNOS to restrain MV$\dot{O}_2$ and eNOS to maintain collateral vasodilation act together to

![Fig. 5. NOS activity in the absence of calcium (iNOS), in the presence of calcium (total NOS activity), and calcium-dependent NOS activity (constitutive NOS). Measurements are from myocardium of normal canine hearts and from the collateral zone and the remote zone of dogs with a collateral-dependent myocardial region. *$P < 0.05$ vs. normal zone myocardium; †$P < 0.05$ vs. collateral zone myocardium.](http://ajpheart.physiology.org/).
prevent the development of ischemia in the collateralized region.

The collateral-dependent region in our experimental model has some characteristics of hibernating myocardium; resting blood flow tended to be decreased despite significant vasodilator reserve, whereas systolic function is reduced (22). Similar to a porcine model of chronic myocardial hibernation (10), we have found that high energy phosphates are preserved in the collateral-dependent region (unpublished data). Although we were unable to sample venous blood from the remote normally perfused region for calculation of \( MV_02 \), during resting conditions oxygen consumption in the collateral-dependent region was 18% less than we previously found in normal hearts (32). This decrease in \( MV_02 \) is similar to that previously reported in the porcine model of chronic myocardial hibernation (10). Our finding that 1400W caused an increase in \( MV_02 \) in the collateral-dependent myocardial region demonstrates that NO produced by iNOS is, at least in part, responsible for the decreased \( MV_02 \), and data suggest that iNOS expression in regions with impaired blood flow may help to preserve myocardial viability by better aligning oxygen demands with the limited blood flow.

Hu et al. (19) demonstrated that hibernating myocardial regions are protected from demand-induced stunning, and this is associated with attenuation of ATP depletion during simulated ischemia, analogous to ischemic preconditioning. They found that respiration was decreased in mitochondria from the hibernating region, suggesting a primary mitochondrial adaptation in the protection from ischemia. Our finding of iNOS expression in the collateralized heart suggests that NO competition with oxygen at cytochrome \( c \) oxidase contributed to the decreased oxygen demands. In addition to effects on mitochondrial respiration, iNOS is implicated in the preconditioning response (14). Interestingly, Hu et al. (19) found that not only was the hibernating region resistant to ischemia, but the remote normally contracting myocardium was also protected. Similarly, we found iNOS expressed not only in the collateralized region but, to a lesser but significant extent, in the remote myocardial region. Ischemia is known to activate multiple pathways that can cause iNOS expression in the heart (17). These responses can account for increased iNOS in the collateral-dependent region but cannot account for iNOS expression and depressed mitochondrial respiration in the remote region. These changes in the remote region are reminiscent of remote preconditioning; such a response at a distance would more likely be explained by circulating or neurogenic factors that could influence both the collateralized and remote regions (19).

We used 1400W in the present study because of its high selectivity for inhibiting iNOS. Thus, in studies using purified iNOS, nNOS, and eNOS protein, 1400W exhibited Ki values of 7 nM, 2 \( \mu \)M, and 50 \( \mu \)M, respectively (28). In rat aortic rings, 1400W was at least 1,000-fold more potent against iNOS as compared with eNOS (13). This high selectivity of 1400W for iNOS makes it unlikely that the results in the present study were related to nonselective blockade of other NOS isoforms.

Limitations. Studies of eNOS and iNOS protein used homogenates of myocardial specimens and so contained both cardiomyocytes as well as vessels. Ideally, we would have liked to study collateral vessels isolated from the myocardium. However, this was not possible because the pericardial reaction after the chronic instrumentation surgery resulted in scarring with adhesion of the pericardium to the heart that made it impossible to identify collateral vessels.

GRANTS

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