Role of Myocardial Neuronal Nitric Oxide Synthase–Derived Nitric Oxide in β-Adrenergic Hyporesponsiveness After Myocardial Infarction–Induced Heart Failure in Rat

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Background—An emerging concept is that a neuronal isoform of nitric oxide synthase (NOS1) may regulate myocardial contractility. However, a role for NOS1-derived nitric oxide (NO) in heart failure (HF) has not been defined.

Methods and Results—Using a model of myocardial infarction-induced HF, we demonstrated that cardiac NOS1 expression and activity increased in HF rats (P<0.05 and P<0.001 versus shams, respectively). This was associated with translocation of NOS1 from the ryanodine receptor to the sarcoplasma through interactions with caveolin-3 in HF hearts. With ex vivo and in vivo pressure-volume analysis, cardiac NOS1-derived NO was found to be negatively inotropic in shams but not HF hearts. Ventricular elastance (Ees) was significantly reduced in HF rats (P<0.05), and τ, the time constant of left ventricular relaxation, was prolonged (both P<0.05). Acute NOS1 inhibition significantly increased Ees by 33±3% and τ by 17±2% (P<0.05) in shams, although these effects were significantly attenuated in HF hearts. β-Adrenergic stimulation induced a marked increase in systolic performance in sham hearts, with the responses being significantly blunted in HF hearts. Ees increased by 163±42% (P<0.01) in sham hearts and 56±9% in HF hearts, and LV +dP/dt increased by 97±9% (P<0.01) in shams and 37±7% (P<0.05) in the HF group. Interestingly, preferential NOS1 inhibition enhanced the blunted responses of LV +dP/dt and Ees to β-adrenergic stimulation in HF rats but had no effect in shams.

Conclusions—These results provide the first evidence that increased NOS1-derived NO production may play a role in the autocrine regulation of myocardial contractility in HF. (Circulation. 2004;110:●●●●●●●.)

Key Words: nitric oxide synthase ■ contractility ■ receptors, adrenergic, β ■ heart failure

The role of NO signaling in the pathophysiology of heart failure (HF) remains controversial. NO-dependent regulation of contractile function is altered in the diseased and failing heart, but the precise abnormalities and the NOS isoforms involved remain to be clarified. Until recently, NOS2 and NOS3 were thought to be the sole isoforms responsible for excessive NO production in the failing heart, leading to cardiac depression in experimental models of HF and patients with end-stage HF both at baseline and after β-adrenergic stimulation.1 However, there is an apparent paradox between the observation that NOS inhibition in HF increases myocardial contractility after β-adrenergic stimulation despite the finding that NOS3-derived NO is decreased in this setting. As such, conclusive data on the underlying mechanisms of NO signaling in HF and the roles of the NOS isoforms involved, both in experimental animal models or humans, remain lacking.1

Previous inconsistent findings may be reconciled by emerging evidence indicating that a neuronal-type NOS1 isoform is present in the cardiomyocyte.2 NOS1 was found to be associated with the cardiac ryanodine receptor Ca2+ release channel (RyR) in the cardiac sarcoplasmic reticulum (SR), which suggests that cardiac NOS1-derived NO may modulate ion channels/transporters involved in myocardial Ca2+ cycling and contraction.3 We have recently demonstrated an upregulation of NOS1 in senescent rat hearts after myocardial infarction4 and in human failing hearts.5 However, a physiological role for NOS1-derived NO in HF has not yet been defined. In the present study, we hypothesize that NOS1-derived NO is increased in the failing myocardium and that this may account, at least in part, for previous conflicting results demonstrating that NOS inhibition increases myocardial contractile responses to β-adrenergic stimulation in HF despite evidence that NOS3-derived NO is decreased. Hence,
the aims of the present study were to investigate whether (1) cardiact NOS1 expression is enhanced in an experimental model of myocardial infarction (MI)–induced HF, (2) NOS1 activity is increased, and (3) NOS1-derived NO plays a significant role in the autocrine regulation of myocardial contractility.

Methods

Animal Model

This study was conducted in accordance with both institutional guidelines and those formulated by the European Community for experimental animal use (L358-86/609/EEC). Three-month-old male Wistar rats (Charles River, France) were subjected to MI by left coronary artery ligation. Sham operation was also performed in age-matched animals. Left ventricular (LV) echocardiography was performed with a General Electric Vivid 7 instrument equipped with a linear 8- to 14-MHz transducer.

NOS Activity, Immunolocalization, Immununoprecipitation, and Coimmunoprecipitation Assays

LV myocardial NOS activity was measured by the conversion of \(^{(3)}H\)-L-arginine (NEN/DuPont) to \(^{(3)}H\)-L-citrulline, as described previously. Assays for specific NOS1 activity were performed in the presence of the preferential NOS1 inhibitor vinyl-L-ornithine (L-VNIO; 0.1 \(\mu\)mol/L), Western blots, immunolocalization, and coimmunoprecipitation were performed with specific antibodies to the following, as described in the online Data Supplement: NOS1, NOS3, caveolin-3 (Cav-3), cardiac ryanodine receptor (a generous gift from Dr I. Marty), vinculin, and \(\alpha\)-actinin.

Assessment of LV Function

Isolated Heart Perfusions

Three months after surgery, rat hearts were perfused with Krebs-Henseleit buffer in nonrecirculating Langendorff mode at constant flow. LV pressure-volume (PV) relationships were obtained with a water-filled polyethylene balloon attached to a pressure transducer inserted into the LV as described (Data Supplement). Acute inhibition of myocardial NOS1 was performed with the preferential NOS1 inhibitor L-VNIO (0.1 \(\mu\)mol/L; \(n=8\) per group). Finally, hearts were subjected to freshly prepared isoproterenol for 5 minutes in the presence or absence of L-VNIO (10\(^{-7}\) mol/L).

In Vivo Integrated Hemodynamic Analysis

Myocardial hemodynamic indices were measured in anesthetized rats with a 2F dual-field combination pressure-conductance catheter (model SPR-819, Millar Instruments) as described (Data Supplement). Measurements were acquired at rest and after dobutamine infusion (at a rate of 5 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\)) in the presence or absence of the preferential NOS1 inhibitor S-Methyl-L-thiocitrulline (SMT) (0.125 mg/kg).

Statistical Analysis

Data are presented as mean±SEM. PV curves for Langendorff-perfused hearts and in vivo experiments, in the presence and absence of NOS1 inhibitor, were compared by 2-way ANOVA for repeated measures followed by the Fisher post hoc test. Other data were analyzed by Student’s unpaired \(t\) test or Student’s paired \(t\) test when comparisons were made before and after perfusion of the NOS inhibitor in the same animal.

Results

Parameters of LV Function

Echocardiographic data demonstrated that systolic and diastolic parameters were significantly altered in HF hearts compared with sham hearts (Data Supplement). Data concerning animal characteristics are given in Table 1.

NOS1 Activity, Expression, and Distribution in HF

Although total calcium-dependent NOS activity in myocardium was similar in sham and HF rats, we noted that NOS3 protein expression was significantly decreased in HF hearts (data not shown), in agreement with previous reports. We were also unable to detect myocardial NOS2 protein or any calcium-independent NOS2 activity (not shown). With L-VNIO (0.1 \(\mu\)mol/L), >70% of total myocardial calcium-dependent NOS activity was found to be attributable to NOS1 in HF animals compared with <30% in shams (Figure 1A; \(P<0.001\)). Western blot analyses confirmed significantly greater expression of NOS1 protein in failing LV homogenates than in shams (Figure 1B; \(P<0.05\)).

Heart sections costained for NOS1 and \(\alpha\)-actinin (a myocyte marker; Figure 1C) confirmed that NOS1 is expressed in the cardiomyocyte. Interestingly, NOS1 immunoreactivity appeared to be significantly increased at the sarcolemmal level in failing hearts, which suggests a partial translocation of NOS1 from the cytosol to the membrane in HF. This was corroborated in sections costained for NOS1 and vinculin (a sarcolemmal marker), which revealed a distinctly sarcolemmal pattern of NOS1 labeling in HF hearts.

To more specifically assess the subcellular localization of NOS1, we studied protein-protein interactions between the NOS isoforms and either Cav-3 or the RyR. Cav-3 protein expression was significantly increased in HF compared with sham hearts (Figure 2A; \(P<0.01\)). Although total protein extracts from sham hearts immunoprecipitated with anti-Cav-3 exhibited minute immunoreactivity with NOS3, the amount of NOS3 protein associated with Cav-3 represented \(>5\%\) of total NOS3 (data not shown). On the other hand, total protein extracts from sham hearts immunoprecipitated with

### Table 1. Animal Characteristics and Echocardiographic Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n=14)</th>
<th>HF (n=14)</th>
<th>(P) (t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>515±20</td>
<td>491±25</td>
<td>NS</td>
</tr>
<tr>
<td>HW, mg</td>
<td>1490±70</td>
<td>2050±80</td>
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</tr>
<tr>
<td>HW/BW</td>
<td>2.93±0.13</td>
<td>4.20±0.35</td>
<td>0.0055</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.98±0.14</td>
<td>4.09±0.33</td>
<td>0.0006</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>339±11</td>
<td>323±19</td>
<td>0.49</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>10.0±2.0</td>
<td>36.6±10.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>71±4</td>
<td>21±8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>9.70±0.04</td>
<td>12.60±0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E, m/s</td>
<td>0.97±0.04</td>
<td>1.53±0.09</td>
<td>0.0002</td>
</tr>
<tr>
<td>A, m/s</td>
<td>0.66±0.06</td>
<td>0.20±0.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E/A</td>
<td>1.5±0.2</td>
<td>7.8±0.3</td>
<td>0.0003</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>18.9±0.9</td>
<td>14.2±0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Ea, cm/s</td>
<td>7.2±0.8</td>
<td>5.5±1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E/eA</td>
<td>14.4±2.0</td>
<td>45.1±4.8</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

BW indicates body weight; HW, heart weight; LVEDP, LV end-diastolic pressure; LVEF, LV ejection fraction; LVEDD, LV end-diastolic diameter; E, peak velocity of early mitral inflow; A, peak velocity of late mitral flow; IVRT, isovolumetric relaxation time; and Ea, early diastolic velocity of mitral annulus.

The presence or absence of the preferential NOS1 inhibitor vinyl-L-ornithine (L-VNIO; 0.1 \(\mu\)mol/L; \(n=8\) per group). Finally, hearts were subjected to freshly prepared isoproterenol for 5 minutes in the presence or absence of L-VNIO (10\(^{-7}\) mol/L).
anti-NOS1 exhibited no immunoreactivity with Cav-3. In the HF group, the amount of NOS1/Cav-3 complexes was dramatically increased, whereas the amount of Cav-3/NOS3 complexes was undetectable (Figure 2B). Finally, immunoprecipitation with anti-RyR in crude particulate preparations revealed significant immunoreactivity with NOS1 in sham hearts, although the amount of these RyR/NOS1 complexes was reduced in HF (Figure 2B). These results are consistent with translocation of NOS1 from the SR to the sarcolemma in HF.

**Physiological Role of NOS1-Derived NO in Cardiac Contractile Function**

*Ex Vivo PV Relationships*

PV relationships in sham and HF hearts are illustrated in Figure 3. Three months after MI, we observed (1) a significant decrease in systolic and LV +dP/dt-volume relationships, (2) an upward shift of the LV −dP/dt-volume relationship, and (3) a rightward displacement of the LV end-diastolic PV curve in HF rats compared with age-matched shams ($P<0.001$ for all PV relationships). L-VNIO induced a small but significant increase in the systolic PV curve in sham hearts ($P<0.05$), whereas this effect was not observed in corresponding HF hearts. The PV relationship for the rate of LV pressure development (LV +dP/dt) was unchanged in the presence of L-VNIO in both sham and HF hearts. The results of the present study also show that exposure of HF hearts to the NOS1 inhibitor L-VNIO caused a significant leftward shift of the LV end-diastolic PV curve toward the LV end-diastolic PV curve observed in control sham hearts ($P<0.05$). Importantly, no change in the LV end-diastolic PV curve was observed in corresponding sham hearts perfused with L-VNIO.
Perfusion with isoproterenol induced a marked increase in LV systolic pressure and peak LV +dP/dt and a decrease in LV −dP/dt in both isovolumic sham and HF hearts; however, these β-adrenergic responses were significantly blunted in HF hearts compared with shams (Figure 4; P<0.01 for all contractile parameters). Interestingly, L-VNIO significantly augmented the mean change in LV systolic pressure, peak LV +dP/dt, and peak LV −dP/dt in response to isoproterenol in HF hearts such that responses resembled those observed in non−L-VNIO−treated sham hearts (P<0.01 for all). In contrast, NOS1 inhibition in sham hearts failed to further increase contractile responses to isoproterenol despite a trend for LV systolic pressure to be decreased (Figure 4).

In Vivo PV Relationships
Figure 5 shows typical PV loops obtained after inferior vena cava occlusion in both groups, and mean data for end-systolic PV relationship are shown in Table 2. Under baseline conditions, hemodynamic measurements demonstrated a significant decrease in the load-independent measure of LV contraction in HF rats (Ees; 0.68±0.08 mm Hg/μL for sham versus 0.25±0.09 for HF rats, P<0.05), which suggests decreased systolic performance in the failing myocardium. Acute pharmacological inhibition of NOS1 (with SMTC) significantly increased Ees by 33±3% and τ by 17±2% in sham hearts, although this effect was blunted in corresponding HF hearts. The same results were obtained for the preload recruitable stroke work, which has been described as independent of chamber size and mass and which is sensitive to changes in contractile function. LV end-diastolic pressure, LV end-systolic volume, and LV end-diastolic volume were significantly increased in HF hearts compared with shams (P<0.01; not shown) and were unaffected by NOS1 inhibition in both groups.

The effects of intravenous dobutamine on LV hemodynamics are reported in Table 2. The positive inotropic response to dobutamine was evident as an increase in the Ees in both groups. Dobutamine increased Ees by 163±42% (P<0.01) in shams but only by 56±9% in the HF group. In addition,
dobutamine increased LV +dP/dt by 97±9% (P<0.01) in shams and 37±7% (P<0.05) in HF rats. Thus, as expected, responses to dobutamine were significantly blunted in HF hearts compared with shams. Importantly, coinfusion of SMTC to inhibit NOS1 rectified the attenuated β-adrenergic response to dobutamine in the HF group, whether assessed by LV +dP/dt or Ees. Indeed, SMTC increased LV +dP/dt by 37±3% (P<0.01) and Ees by 77±15% (P<0.05) in HF rats. Similar effects of NOS1 inhibition were observed for preload recruitable stroke work. In accordance with the ex vivo experiments, coinfusion of SMTC failed to further increase the contractile response to dobutamine in sham hearts. Hemodynamic measurements showed a trend for LV end-diastolic pressure to be higher in the HF group after coinfusion of SMTC, without affecting LV end-diastolic volume (not shown), consistent with decreased diastolic distensibility. In both groups, coinfusion of the NOS1 inhibitor also had no effect on LV end-systolic volume.

Coupling of ventricular elastance to arterial elastance (Ees/Ea) was significantly decreased in HF hearts, which indicates that LV function of HF rats had a decreased mechanical efficiency compared with the hearts of sham rats (Table 2). Dobutamine induced a marked increase in the Ees/Ea ratio in both sham and HF hearts. However, these β-adrenergic responses were significantly blunted in HF hearts compared with shams. Interestingly, SMTC significantly augmented both the Ees/Ea ratio and the end-diastolic volume in response to isoproterenol only in HF hearts, which suggests that NOS1 inhibition improved LV coupling and increased work efficiency.

**Discussion**

The principal novel finding of this study is that rats with MI-induced HF exhibit increased myocardial sensitivity to β-adrenergic stimulation after pharmacological NOS1 inhibition, as indicated by increased cardiac contractile responses. These functional changes in the HF group are associated with an increase in Cav-3 abundance and both cardiac NOS1 expression and activity. We further demonstrated that HF is associated with a precise spatial confinement of NOS1 to the sarcolemma, where the enzyme interacts with Cav-3. The increased amounts of NOS1/Cav-3 complexes in HF may constitute an increased pool of enzyme available for activation on stimulation. Moreover, a decrease in the level of RyR/NOS1 complexes was observed in the HF group, which also indicates a change in the subcellular compartmentalization of NOS1. Thus, an increase in the concentration of the caveolar scaffolding protein, Cav-3, that compartmentalizes NOS1 with stimulatory agonist-signaling molecules/receptors suggests a novel mechanism by which NOS1 pathway activity may be increased in HF and supports the concept that NOS1-derived NO may distinctively modulate myocardial inotropy in HF.

**Effect of NOS1-Derived NO on Myocardial Contractility**

Although previous studies assumed that cardiac NOS3 was the sole source of NO involved in the autocrine regulation of myocardial contraction and Ca2+ homeostasis, emerging evidence indicates that NOS1 located in the cardiac SR may play a significant role in modulating cardiac contractility by regulating Ca2+ fluxes. The present study revealed that pharmacological inhibition of NOS1 increased myocardial contraction and slowed myocardial relaxation in normal...
TABLE 2. Hemodynamics Measured With Conductance Catheter

<table>
<thead>
<tr>
<th></th>
<th>Sham Basal</th>
<th>Sham Basal + SMTC</th>
<th>Sham Dobutamine</th>
<th>HF Basal</th>
<th>HF Basal + SMTC</th>
<th>HF Dobutamine + SMTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>257±24</td>
<td>246±17</td>
<td>342±11*</td>
<td>239±12</td>
<td>232±13</td>
<td>316±18</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>115±8</td>
<td>136±12</td>
<td>138±6*</td>
<td>103±4</td>
<td>113±13</td>
<td>114±4†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>10±2</td>
<td>11±3</td>
<td>13±2</td>
<td>25±5†</td>
<td>29±4</td>
<td>18±4†</td>
</tr>
<tr>
<td>Ees, mm Hg/mL</td>
<td>0.76±0.23</td>
<td>0.97±0.28</td>
<td>0.93±0.36</td>
<td>0.65±0.32</td>
<td>0.80±0.27</td>
<td>0.75±0.24</td>
</tr>
<tr>
<td>ESV, mm Hg/s</td>
<td>6064±830</td>
<td>6992±775</td>
<td>11 991±872*</td>
<td>4960±438†</td>
<td>4796±533</td>
<td>6810±816†</td>
</tr>
<tr>
<td>dP/dt, mm Hg/s</td>
<td>5342±551</td>
<td>7256±251†</td>
<td>6604±780†</td>
<td>3663±163†</td>
<td>3008±215</td>
<td>4618±436†</td>
</tr>
<tr>
<td>τ, ms</td>
<td>17.5±1.5</td>
<td>20.5±1.1†</td>
<td>15.5±0.5‡</td>
<td>25.2±2.9†</td>
<td>27.3±6.5</td>
<td>19.0±1.8†</td>
</tr>
<tr>
<td>Ees, mm Hg/µL</td>
<td>0.68±0.08</td>
<td>0.90±0.09†</td>
<td>1.79±0.25*</td>
<td>0.25±0.09†</td>
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<td>PRSW, mm Hg</td>
<td>80.8±12.5</td>
<td>119.3±14.3‡</td>
<td>117.5±16.0‡</td>
<td>45.4±3.0†</td>
<td>51.3±6.0</td>
<td>69.3±8.6†</td>
</tr>
<tr>
<td>dP/dt-EDV, mm Hg·s⁻¹·µL⁻¹</td>
<td>24.8±3.9</td>
<td>42.2±6.3‡</td>
<td>53.5±13.0‡</td>
<td>17.9±2.4</td>
<td>16.3±7.6</td>
<td>16.1±4.5</td>
</tr>
<tr>
<td>EDV, µL</td>
<td>241±46</td>
<td>253±17</td>
<td>348±58</td>
<td>744±87</td>
<td>774±106</td>
<td>647±40</td>
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<td>ESV, µL</td>
<td>117±41</td>
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<td>180±30</td>
<td>637±35</td>
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<tr>
<td>Ees/Ea</td>
<td>0.68±0.39‡</td>
<td>0.92±0.27</td>
<td>1.93±0.63‡</td>
<td>2.22±0.62</td>
<td>0.33±0.10</td>
<td>0.53±0.09†</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; Ees, arterial elastance; LV +dP/dt, maximum rate of LV pressure rise; LV –dP/dt, maximal rate of LV pressure fall; τ, time of isovolumic relaxation; Ees, ventricular elastance; PRSW, preload recruitable stroke work; dP/dt-EDV, LV +dP/dt normalized to end-diastolic volume; EDV, end-diastolic volume; and ESV, end-systolic volume.

Data are mean±SEM (n=8 per group).

*P<0.01 vs basal.
†P<0.05 vs sham.
‡P<0.05 vs basal.
§P<0.05 vs dobutamine.
¶P<0.01 vs sham.
‖P<0.01 vs dobutamine.

Figure 5. Representative LV PV loops and end-systolic PV relationships (ESPVR), obtained by transient inferior vena cava occlusion, at baseline, in response to dobutamine (Dob), and in response to dobutamine in presence of NOS1 inhibitor SMTC in sham and HF rats (n=8 per group). HF rats demonstrate attenuated contractile response at baseline and in response to dobutamine (contractility indexed by slope position of Ees; upward deflection signifies positive inotropy). However, pharmacological inhibition of NOS1 by SMTC caused β-adrenergic responses to dobutamine to significantly increase in HF group, whereas they remained unchanged in sham animals.
NOS inhibition in HF increases myocardial contractility after β-adrenergic stimulation despite the finding that NOS3-derived NO is decreased in this setting. Indeed, using both ex vivo and in vivo approaches, we provide evidence that myocardial contractility is significantly improved when NOS1 activity is blocked by a preferential NOS1 inhibitor in rats with HF.

Furthermore, failing hearts exposed to NOS1 inhibition demonstrated reduced cavity dilation and a significant leftward shift of the LV end-diastolic PV curve toward that observed in sham animals. In the same way, NOS1 inhibition in vivo tended to increase LV end-diastolic pressure without affecting LV end-diastolic volume in the failing heart. This suggests that in addition to influencing contractility, NOS1 enhanced LV chamber compliance in HF. These findings are consistent with previous work performed in experimental preparations and in the failing human heart indicating that NO may increase LV diastolic distensibility. An increase in cardiomyocyte diastolic length induced by NO donors or 8-bromo-cGMP in the absence of changes in diastolic Ca2+ has been suggested to reflect an acute reduction in diastolic tone and is analogous to the changes in LV end-diastolic PV curve relationships observed in clinical studies.

The underlying mechanism of this change may involve reduced force generation by low-intensity diastolic crossbridge cycling (secondary to reduced myofilament Ca2+ responsiveness), alterations in cytoskeletal protein properties, or a cGMP-mediated change in myocyte cell volume.

**Myocardial NO Signaling in Heart Failure: Role of Cav-3 in NOS1 Signaling**

The present data support the concept of a differential role of NOS1-derived NO at baseline and during β-adrenergic stimulation. Indeed, pharmacological NOS1 inhibition increased basal cardiac contractility in sham hearts but not in failing hearts. Conversely, whereas NOS1 inhibition led to a significant increase in responses to β-adrenergic stimulation in failing hearts, it had no effect in shams. The decrease or loss of NOS1 pathway activity under basal conditions in animals with HF is consistent with a potential increase in an inhibitory factor, such as caveolin. Caveolins are scaffolding proteins found in caveolae, which are plasmalemmal microdomains that participate in signal transduction by means of colocalizing membrane receptors (eg, β-adrenoceptors) with signal transduction effectors. Because caveolin inhibits NOS activity by preventing calmodulin activation, it may exert dual regulation of NOS in HF, ie, inhibition of basal activity yet augmentation of agonist-stimulated actions. In support, in a recent study using a model of canine HF, the authors suggest that the increased levels of Cav-3 observed may contribute to enhanced NO signaling (ie, altered β-adrenergic responses) via a compartmentation effect, whereas basal unstimulated NO activity might be depressed. Indeed, Hare et al observed that nonselective NOS inhibition reduced basal myocardial contractility in control dogs but had no effect in dogs with congestive HF, similar to recent results in humans. It has also been demonstrated that NOS1 activity is influenced by its reciprocal interaction with Cav-3. Given these findings, we explored the hypothesis that this endogeneous regulator of NOS1 activity might be altered in HF. We revealed that (1) Cav-3 protein expression is increased in the failing myocardium, and (2) HF is associated with a precise spatial confinement of Cav-3 to the sarcolemma, where it interacts with Cav-3. This increased amount of NOS1 associated with Cav-3 could constitute an increased pool of enzyme that is available for activation on stimulation (eg, β-adrenergic agonists). The present observations that there is increased protein expression of NOS1 and Cav-3 in HF, together with increased translocation of NOS1 to the sarcolemma, where it interacts with Cav-3, may account, at least in part, for the previously observed effects of nonselective NOS inhibition classically observed.

**Spatial Confinement of NOS1 in Cardiac Myocytes: Role in HF**

In cardiac myocytes, NOS1 localizes in the SR, the organelle responsible for the intracellular calcium cycle that drives excitation-contraction coupling (EC coupling) and, in turn, the cardiac cycle. Indeed, it is now well established that NOS1-derived NO regulates RyR in the SR by fine-tuning receptor activity. Indeed, exogenous NO can directly regulate RyR activity (via nitrosylation), which has been shown to be both activated and inhibited, although NOS1 was not directly implicated. Recently, Barouch et al hypothesized that NOS1-derived NO would facilitate myocardial contractility by enhancing Ca2+ cycling. In support of this hypothesis, they demonstrated stimulatory influences of NOS1-derived NO on both β-adrenergic inotropic responses and Ca2+ transients using NOS1−/− mice, but without altering L+. Although several groups now agree that cardiac myocyte contains NOS1 and that it influences Ca2+ cycling, the details of this regulation remain highly controversial. A complicating aspect here is that RyRs are very closely colocalized with L-type Ca2+ channels. Thus, given the rapid diffusion of NO, it is difficult to envision how NOS1-derived NO can affect RyRs without also altering L+. Also, using the same NOS1−/− mice, Ashley et al found opposite results (increased basal contractility and β-adrenergic responsiveness) and increased SR Ca2+ load. Discrepancy between the 2 groups may be technical (temperature, frequency, degree of β-adrenergic stimulation) or related to the age (or hypertrophy stage) at which animals were studied.

The implications of the results of the present study could be far-reaching because of the dual consequences: increased membrane NOS1 activity in caveolae and decreased NOS1 activity in the SR. Indeed, loss of local NO in the SR depresses both systolic and diastolic reserve in intact animals. Increased production of NO in caveolae could intensify inhibition of L-type Ca2+ channel, further impairing myocardial contractility. Membrane NOS1 is also linked to the plasmalemmal Ca2+ pump in the caveolae, although the consequences of this association in the cardiomyocyte remain unknown. Although we did not address the role of NOS1-derived NO in RyR activity, both the demonstration of RyR/NOS1 complexes and the increased inotropic effect of SMTC in sham hearts are in agreement with a potential role for NOS1 in the regulation of SR Ca2+ load in nondiseased hearts. Moreover, the observation that NOS1 inhibition elic-
ited a no inotropic response under basal conditions in failing hearts suggests that NOS1 activity under these conditions is reduced, consistent with the decreased levels of RyR/NOS1 complexes observed in the HF group. The idea that NOS1 signaling is spatially confined raises the question of consequences in downstream signaling. At least 2 mechanisms for NO signaling are known24: posttranslational modification of proteins via S-nitrosothiol bond formation and cGMP production. Thus, specificity in NO signaling might arise from specific protein-protein interactions between NOS1 and signaling effectors and from use of different signaling effectors in specific organelles. This hypothesis needs further testing but could have major implications for understanding NO signaling in the cardiomyocyte.

Study Conclusions

The present study demonstrates for the first time that rats with HF exhibit increased sensitivity to β-adrenergic stimulation after preferential NOS1 inhibition. However, in view of recent findings reporting a novel mechanism by which intracellular Ca2+ is regulated by NOS1-derived NO in LV myocytes, the in vitro role of cardiac NOS1-derived NO on calcium handling and myofilament sensitivity in HF, a pathology characterized by electromechanical dysfunction characterized by electromechanical dysfunction and calcium-handling abnormalities, requires further development.

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