Systemic Inhibition of Nitric Oxide Synthase Unmasks Neural Constraint of Maximal Myocardial Blood Flow in Humans

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Background—Nitric oxide (NO) is an endothelial mediator that regulates vascular smooth muscle tone, but it may exert its cardiovascular action also by modulating the autonomic control of vasomotor tone. We assessed the effect of simultaneous inhibition of both endothelial (eNOS) and neuronal (nNOS) NO synthase isoforms on myocardial blood flow (MBF) and coronary flow reserve (CFR) in volunteers and in (denervated) transplant recipients.

Methods and Results—MBF (mL·min⁻¹·g⁻¹) was measured at rest and during adenosine-induced hyperemia with positron emission tomography and ¹⁵O-labeled water. CFR was calculated as adenosine/resting MBF. Measurements were repeated during one of the following intravenous infusions: group 1 (n=12), saline; group 2 (n=9), 3 mg/kg N⁶-monomethyl-L-arginine (L-NMMA), which crosses the blood-brain barrier and inhibits both eNOS and nNOS; group 3 (n=13), 10 mg/kg L-NMMA; group 4 (n=8), phenylephrine titrated to simulate the hemodynamic changes in group 3; and group 5 (n=6), 10 mg/kg L-NMMA infused into the heart transplant recipients. After intervention, hyperemic MBF and CFR were unchanged in groups 1, 2, and 4. By contrast, both hyperemic MBF (+53%, P<0.0001 versus baseline) and CFR (+52%, P<0.0001 versus baseline) increased in group 3, whereas they remained unchanged in group 5, which suggests that an intact cardiac innervation was required for the increase in MBF and CFR observed in group 3.

Conclusions—The results of the present study suggest that maximal adenosine-induced hyperemia and CFR in humans are constrained by neurally mediated vasoconstriction, which can be relieved by systemic NOS inhibition with L-NMMA.


Key Words: nitric oxide ■ nitric oxide synthase ■ blood flow ■ imaging

Nitric oxide (NO) has been primarily recognized as an endothelial mediator that directly regulates vascular smooth muscle tone. Accordingly, it has been shown that intracoronary administration of the NO synthase (NOS) inhibitor N⁶-monomethyl-L-arginine (L-NMMA) reduces epicardial coronary artery diameter and blood flow through inhibition of endothelial NOS (eNOS).¹,² Growing evidence, however, suggests that NO may also exert its action on the coronary circulation indirectly by modulating the autonomic control of vasomotor tone.³ A recent noninvasive study in humans has demonstrated that an intact cardiac innervation is required for the physiological vasodilator response of the coronary circulation during cold pressor test.⁴

Although the exact role of NO in this context remains to be defined, previous in vivo and ex vivo studies in animals have supported the concept that NO derived from neuronal NO synthase (nNOS) contributes to the regulation of vasomotor tone and blood pressure.⁵-⁹ It has also been demonstrated that NO produced by neurons in the central nervous system acts as a neurotransmitter, the primary effect of which is to constrain sympathetic excitability.⁶-¹³ Intravenous infusion of the NOS inhibitor L-NMMA in both animals and humans has been shown to increase overall efferent sympathetic activity, although the associated rise in blood pressure would be expected to induce a baroreflex-mediated decrease in sympathetic tone.⁸-¹⁴ This effect of intravenous L-NMMA infusion on efferent sympathetic activity has been attributed to its central inhibition of nNOS because L-NMMA crosses the blood-brain barrier. The inhibition of central nNOS, in turn, would lessen the physiological constraint exerted by NO on sympathetic output.¹⁴

In the present study, we aimed to assess the net effect of simultaneous inhibition of both NOS isoforms on myocardial blood flow (MBF) and coronary flow reserve (CFR) measured noninvasively by positron emission tomography (PET).
We hypothesized that systemic L-NMMA infusion would inhibit central nNOS, thus disinhibiting efferent sympathetic activity, which in turn would increase CFR in healthy human volunteers but not in heart transplant recipients because of their cardiac denervation.

**Methods**

**Study Population**

We studied 42 healthy male volunteers (age range 35 to 60 years). None of them had a history of cardiovascular disease, smoking, or any other cardiovascular risk factor. Accordingly, none of the volunteers was receiving any form of treatment. Enrollment criteria included normal heart rate, blood pressure, ECG, and 2D echocardiogram and low clinical probability of coronary artery disease.15 In all volunteers, CFR measured by PET was within normal limits in all volunteers but not in heart transplant recipients because of their cardiac denervation.

**Measurement of MBF**

MBF was measured with $^{15}$O-labeled water (H$_2^{15}$O) and an ECAT 931-08/12 15-slice PET scanner (CTI/Siemens). H$_2^{15}$O (700 to 900 MBq) was injected as an intravenous bolus over 20 seconds at an infusion rate of 10 mL/min, and dynamic scanning was acquired over a period of 5.5 minutes.18

The sinograms obtained were corrected for attenuation and reconstructed on a MicroVax II computer (Digital Equipment Corp) with dedicated array processors and standard reconstruction algorithms.

**CFR and Coronary Resistance**

CFR was calculated as the ratio of adenosine to resting MBF. To account for the variability of coronary driving pressure, resting and minimal (ie, during adenosine infusion) coronary resistance (mm Hg · mL$^{-1}$ · min$^{-1}$ · g$^{-1}$) was also calculated as the ratio of mean arterial pressure to MBF.$^{25,26}$

**Statistical Analysis**

Data are reported as mean±SD. Statistical comparisons of hemodynamic data, MBF, CFR, and coronary resistance during the different study conditions were performed by ANOVA for repeated measure.

**TABLE 1. Subject Characteristics**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, y</th>
<th>Body Mass Index, kg/m$^2$</th>
<th>Total Cholesterol, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>46±6</td>
<td>26±3</td>
<td>5.4±1.3</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>45±8</td>
<td>27±3</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>44±7</td>
<td>26±2</td>
<td>5.2±0.9</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>49±8</td>
<td>27±3</td>
<td>5.3±1.1</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>57±2</td>
<td>28±5</td>
<td>5.3±0.8</td>
</tr>
</tbody>
</table>
after infusions were comparable to their respective baseline through 4 both at baseline and after infusion of L-NMMA. In resting MBF was significantly higher than in groups 1—Table 3. In patients who had received transplants (group 5), MBF, CFR, and Coronary Resistance

MBF, CFR, and Coronary Resistance

MBF and CFR data for each study group are presented in Table 3. In patients who had received transplants (group 5), resting MBF was significantly higher than in groups 1 through 4 both at baseline and after infusion of L-NMMA. In groups 1, 2, 4, and 5, resting and hyperemic MBF and CFR after infusions were comparable to their respective baseline values. Similar to the other groups, resting MBF was unaffected by the infusion of LNMMA in group 3. By contrast, hyperemic MBF and CFR increased significantly after the infusion of the high dose (10 mg/kg) of L-NMMA compared with the baseline value and with the values in groups 1, 2, 4, and 5 after infusions. However, when the same high dose of L-NMMA was administered to the patients who had received transplants, no changes in MBF and CFR were observed compared with baseline (Figure 2).

At baseline, coronary resistance was comparable in the 5 groups both at rest and during adenosine infusion (data not shown). The changes in minimal coronary resistance after the infusions are illustrated in Figure 3. After the infusion of 10 mg/kg L-NMMA, a further decrease (−35%, P <0.0005) was observed in group 3, whereas there was a nonsignificant trend toward an increase in group 5.

Discussion

The present study demonstrates that intravenous administration of L-NMMA at 10 mg/kg in healthy volunteers increases adenosine-induced hyperemia by ≈50%. This effect appears to necessitate an intact cardiac innervation, because no increase was observed in transplant recipients (6.5±2 months after transplantation), whose hearts remain totally denervated for >1 year after the operation.16

Our findings, that L-NMMA did not decrease resting MBF while increasing hyperemic MBF and CFR, are only appar-

TABLE 2. Hemodynamics

<table>
<thead>
<tr>
<th>GR</th>
<th>MAP</th>
<th>HR</th>
<th>RPP</th>
<th>MAP</th>
<th>HR</th>
<th>RPP</th>
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<tbody>
<tr>
<td>1</td>
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<td>62±6</td>
<td>7615±1559</td>
<td>86±12</td>
<td>93±15</td>
<td>11 584±2842</td>
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<tr>
<td>2</td>
<td>86±9</td>
<td>57±9</td>
<td>6566±1062</td>
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<td>60±9</td>
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<td>11 741±1968</td>
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<tr>
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<td>88±13</td>
<td>60±11</td>
<td>7278±1797</td>
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<td>84±16</td>
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<td>92±14</td>
<td>12 576±1597</td>
<td>98±10</td>
<td>95±13</td>
<td>12 566±1735</td>
</tr>
</tbody>
</table>

GR indicates group; HR, heart rate (bpm); MAP, mean arterial pressure (mm Hg); and RPP, rate pressure product (systolic blood pressure×HR). *Intervention was one of the following infusions according to the study protocol: in group 1, saline; in group 2, 3 mg/kg L-NMMA IV; in group 3, 10 mg/kg L-NMMA; in group 4, phenylephrine; and in group 5, 10 mg/kg L-NMMA.

†P<0.05, ‡P<0.005 vs respective baseline. §P<0.05 vs all other groups.

Results

All procedures were well tolerated apart from the common side effects caused by adenosine, such as flushing and chest tightness. None of the subjects experienced any symptoms or ECG changes after infusion of L-NMMA or phenylephrine.

Hemodynamics

At baseline, heart rate and mean arterial blood pressure at rest and during adenosine were higher in group 5. Hemodynamic parameters remained unchanged in groups 1 and 2 after the infusions. By contrast, mean arterial pressure was significantly increased in groups 3 (+10±1%; P<0.05), 4 (+11±1%; P<0.05), and 5 (+11±1%; P<0.05) compared with baseline and compared with groups 1 and 2. This was accompanied by a decrease in heart rate in groups 3 (−13±1%; P<0.05) and 4 (−13±1%; P<0.05) but not in group 5 (±2±3%; P=NS; Table 2).

MBF, CFR, and Coronary Resistance

TABLE 3. MBF and CFR

<table>
<thead>
<tr>
<th>GR</th>
<th>n</th>
<th>Baseline</th>
<th>Intervention*</th>
<th>Baseline</th>
<th>Intervention*</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Rest</td>
<td>Adenosine</td>
<td>Rest</td>
<td>Adenosine</td>
<td></td>
</tr>
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<td>12</td>
<td>0.89±0.11</td>
<td>3.26±0.62</td>
<td>0.99±0.11</td>
<td>3.65±0.38</td>
<td>2.79±0.76</td>
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<tr>
<td>2</td>
<td>9</td>
<td>0.78±0.12</td>
<td>3.42±0.74</td>
<td>0.81±0.06</td>
<td>3.51±0.64</td>
<td>2.91±0.77</td>
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<tr>
<td>3</td>
<td>13</td>
<td>0.88±0.13</td>
<td>3.40±0.94</td>
<td>0.90±0.17</td>
<td>5.23±0.64</td>
<td>2.65±0.67</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.81±0.08</td>
<td>3.31±0.87</td>
<td>0.81±0.17</td>
<td>3.65±0.92</td>
<td>2.93±0.79</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>1.31±0.19</td>
<td>3.08±0.28</td>
<td>1.33±0.22</td>
<td>2.76±0.65</td>
<td>2.43±0.56</td>
</tr>
</tbody>
</table>

GR indicates group. *Intervention: infusion according to protocol.

†P<0.05, ‡P=0.0001 vs baseline; §P<0.05, ||P<0.005 vs all other groups.
ently in contradiction with a number of previous studies showing a decrease in MBF after administration of L-NMMA. In fact, in those studies, L-NMMA was infused directly into the coronary circulation at doses that block coronary eNOS but without any significant systemic hemodynamic effect.

The vasodilator effect of adenosine was originally thought to be based solely on the direct stimulation of A2 adenosine receptors on vascular smooth muscle cells, which mediate an increase in the second-messenger cAMP by stimulating adenylate cyclase. Therefore, this agent has been used frequently in animal and human studies to evaluate endothelium-independent vasodilation. However, in the past decade, it has been appreciated that adenosine also acts, at least in part, as an endothelium-dependent vasodilator, both via flow-mediated dilation and via direct stimulation of endothelial cells.

We infused L-NMMA intravenously at a dose that has been shown to increase blood pressure and activate the baroreflex and to effectively block eNOS and nNOS in experimental animals and humans. Given that the coronary circulation extracts ~5% of cardiac output, with our systemic doses of L-NMMA, we would expect to achieve coronary concentrations of ~50 μmol (group 2) and 164 μmol (groups 3 and 5), respectively, which compare well with the figures of 32, 125, and 320 μmol shown to block coronary eNOS when administered via an intracoronary route. Thus, it is reasonable to assume that with our high-dose L-NMMA infusion, we have successfully blocked a substantial amount of both eNOS and nNOS. Although we cannot distinguish the isolated contribution of each NOS isoform inhibition, we can assume that the difference between local versus systemic infusion of L-NMMA must be due to the additional nNOS inhibition, resulting in a net increase in flow.

We did not observe any change in resting MBF after intravenous infusion of 3 or 10 mg/kg L-NMMA. This is in apparent contradiction with previous reports, which found a decrease in cross-sectional area and coronary flow after intracoronary L-NMMA administration. However, there is no study in the literature in which resting MBF was measured before and after intravenous L-NMMA infusion. Buus et al found a decrease in adenosine-induced hyperemia after intravenous infusion of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), which does not cross the blood-brain barrier. Resting MBF was not assessed. In all other studies in which a decrease in resting MBF after L-NMMA administration was observed, L-NMMA was injected via intracoronary routes. The only case in which a dose of L-NMMA similar to the one used by us was injected systemically is the study by Owlya et al, in which MBF was not measured. They found, however, an increase in efferent muscle sympathetic nerve activity that supports our interpretation of the data of the present investigation.

Our 2-step dose-finding study revealed a different effect of the low (3 mg/kg) and high (10 mg/kg) doses of L-NMMA on adenosine-induced hyperemia. The reasons for the lack of any response at the low L-NMMA dose are not clear, although a different ED50 for L-NMMA–induced blockade of eNOS and nNOS has been suggested. Alternatively, inhibition of the

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**Figure 2.** Influence of intravenous infusion of 10 mg/kg L-NMMA on individual resting and hyperemic MBF in healthy volunteers and heart transplant recipients. L-NMMA significantly increased hyperemic MBF in volunteers but not in patients who had received transplants.

**Figure 3.** Minimal coronary resistance during adenosine. In group 1, measurement before and after saline infusion revealed highly comparable values, which confirmed excellent repeatability of minimal coronary resistance assessment by PET. Infusion of 3 mg/kg L-NMMA did not change resistance, whereas infusion of 10 mg/kg L-NMMA led to a significant decrease in minimal resistance. This was not due to the increase in blood pressure because titration of phenylephrine to same target blood pressure increase had no influence on minimal coronary resistance. In patients who had received transplants, minimal coronary resistance tended to increase after L-NMMA.
tonic central neuronal constraint on sympathetic outflow may be too limited at low L-NMMA doses to counterbalance the local effect of eNOS inhibition, whereas this balance might be shifted at high L-NMMA doses. It is also possible that the lower dose simply might have been too low to induce any effect.

In contrast to the lower L-NMMA dose, there was a significant increase in blood pressure after infusion of the high dose. This was paralleled by a baroreflex-mediated decrease in heart rate in healthy volunteers but not in transplanted subjects, which is a further confirmation that their hearts were still denervated. The increase in perfusion pressure cannot explain the observed increase in hyperemic MBF and CFR as in group 4, where phenylephrine was titrated to achieve an increase in blood pressure similar to that obtained in group 3; in fact, MBF and CFR were unaffected by this change in pressure. This is in line with previous results with intracoronary Doppler catheter measurements\(^8\) that showed no change in baseline and hyperemic MBF assessed during intravenous phenylephrine infusion (targeting a 30-mm Hg blood pressure increase).

It has been suggested that NO may, in addition to its direct vasodilator action, exert its cardiovascular actions by direct chronotropic effects\(^9\) and by modulating sympathetic vasoconstrictor tone.\(^{4,40-42}\) It remains controversial whether inhibition of nNOS elicits sympathoexcitatory effects\(^14\) or not,\(^{37}\) because it may exert opposing effects at different sites.\(^{43}\) The present study is the first to report a net increase in adenosine-induced hyperemic MBF in response to L-NMMA. Our results suggest that this is mainly due to central nNOS inhibition, which leads to increased sympathoexcitatory efferents that overrule local eNOS blockade.

The finding that pharmacologically induced vasodilation may lead to submaximal MBF is consistent with our previous observation that dipyridamole-induced hyperemia can increase by \(\approx40\%\) after administration of a selective \(\alpha_1\)-adrenoceptor blocker.\(^{38}\) We hypothesize that the reflex sympathoexcitatory activation elicited after the systemic administration of vasodilators such as adenosine and dipyridamole would result in a further fall in minimal coronary resistance, which, however, is blunted by an NO-modulated suppression of sympathetic outflow in the central nervous system. This is in line with our previous study,\(^{26}\) which showed that an increase in the standard adrenaline rate of 140 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) does not further increase maximal MBF. The present results suggest that when the vasodilator is administered during systemic inhibition of NOS, this constraint is removed, and the overall effect is a further dilatation and a higher hyperemic flow. The further fall in minimal resistance could be due to activation of \(\beta_1\)-adrenoceptors on coronary arterioles, as recently reported by Sun and coworkers.\(^{44}\) The findings by Buus et al\(^{33}\) showing that inhibition of NOS by L-NAME elicits \(\alpha_1\)-adrenergic–mediated vasoconstriction, which limits adenosine-induced hyperemia, are only in apparent contradiction with the present data. It is worth pointing out that L-NAME does not cross the blood-brain barrier. Therefore, the experimental conditions are equivalent to the transplant recipients in the present study, in whom we observed an analogous trend toward reduced CFR after NOS inhibition. Although in 30% of transplant recipients an upregulation of inducible NOS has been found to be associated with decreased CFR (\(<2\)),\(^{45}\) we believe that this has little impact on the present results because, first, L-NMMA blocks all NOS isoforms, and second, only 1 transplant recipient had a CFR below 2.

In conclusion, the results of the present study suggest that maximum adenosine-induced hyperemic MBF and CFR in humans are constrained by neurally mediated vasoconstriction. The latter can be relieved by systemic NOS inhibition with L-NMMA.

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References


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