Systemic nitric oxide synthase inhibition improves coronary flow reserve to adenosine in patients with significant stenoses

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Kaufmann PA, Rimoldi OE, Gnocchi-Ruscone T, Luscher TF, Camici PG. Systemic nitric oxide synthase inhibition improves coronary flow reserve to adenosine in patients with significant stenoses. Am J Physiol Heart Circ Physiol 293: H2178–H2182, 2007. First published July 27, 2007; doi:10.1152/ajpheart.01292.2006.—We studied the impact of systemic infusion of the nitric oxide synthase (NOS) inhibitor Nω-monomethyl-L-arginine (L-NMMA) on coronary flow reserve (CFR) in patients with coronary artery disease (CAD). We have previously demonstrated that CFR to adenosine was significantly increased after systemic infusion of L-NMMA in normal volunteers but not in recently transplanted denervated hearts. At baseline, myocardial blood flow (MBF; ml·min−1·g−1) was measured at rest and during intravenous administration of adenosine (140 μg·kg−1·min−1) in 10 controls (47 ± 5 yr) and 10 CAD patients (58 ± 8 yr; P < 0.01 vs. controls) using positron emission tomography and 15O-labeled water. Both MBF measurements were repeated during intravenous infusion of 10 mg/kg L-NMMA. CFR was calculated as the ratio of MBF during adenosine to MBF at rest. CFR was significantly higher in healthy volunteers than in CAD patients and increased significantly after L-NMMA in controls (4.00 ± 1.10 to 6.15 ± 1.35; P < 0.0001) and in patients, both in territories subtended by stenotic coronary arteries (>70% luminal diameter: 2.06 ± 1.13 to 3.21 ± 1.07; P < 0.01) and in remote segments (3.20 ± 1.23 to 3.92 ± 1.62; P < 0.05). In conclusion, CFR can be significantly increased in CAD by a systemic infusion of L-NMMA. Similarly to our previous findings in normal volunteers, this suggests that adenosine-induced hyperemia in CAD patients is constrained by a mechanism that can be relieved by systemic inhibition with L-NMMA.

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In a recent study in normal volunteers (15), we demonstrated that hyperemic MBF measured during the combined intravenous infusion of adenosine (140 μg/kg) and the nitric oxide synthase (NOS) inhibitor Nω-monomethyl-L-arginine (L-NMMA; 10 mg/kg) was 53% higher than that during infusion of adenosine alone. This effect seems to necessitate an intact cardiac innervation, since no increase in MBF during the combined intravenous infusion of adenosine and L-NMMA was observed in transplant recipients (6.5 ± 2 mo after transplant) whose hearts remain denervated for more than a year after the grafting (1).

The purpose of the present study was to assess whether a limitation of adenosine-induced hyperemia, similar to that previously demonstrated in normal volunteers, is present in patients with coronary artery disease (CAD).

METHODS

Study population. Ten patients (1 female) age 58 ± 8 yr with single-vessel CAD were studied. Mean total cholesterol was 6.1 ± 0.8 mM. All patients described at least a 3-mo history of chronic stable angina pectoris and had electrocardiographic (ECG) evidence of myocardial ischemia, defined as ≥0.1 mV of horizontal or downsloping ST segment depression, during exercise stress. All patients had a significant stenosis (≥70% of luminal diameter) in a major coronary branch. The coronary angiograms were analyzed by two independent, experienced operators. The luminal diameter of the stenosed artery and adjacent reference lumen was measured at end diastole in the projection that demonstrated the most severe stenosis, and quantitative analysis was performed using an automated edge-contour detection system (Centricity QCA; General Electric Medical Systems, Milwaukee, WI). A significant stenosis was found in the left anterior descending artery in five patients, in the left circumflex in one patient, and in the right coronary artery in four patients. All patients were receiving treatment with aspirin and β-blocking agents. However, in all patients β-blockers were withdrawn 48–72 h before the study day. Exclusion criteria were a recent history (<3 mo) of myocardial infarction or unstable angina, an inability to undergo exercise tolerance testing, or resting ECG patterns that would interfere with interpretation of ST changes during exercise.

A group of 10 healthy male volunteers age 47 ± 5 yr (P < 0.01 vs. patients) served as controls. The lipid profile was assessed in all volunteers, and those with total cholesterol ≥6.4 mM (250 mg/100 ml) were excluded from the study. Their total cholesterol was 5.2 ± 1.0 mM. None of them had a history of cardiovascular disease, smoking, or any other cardiovascular risk factor. None of the volunteers was receiving any form of drug treatment. Enrollment criteria included normal heart rate, blood pressure, ECG, two-dimensional coronary circulation; autonomic nervous system; ischemic heart disease; cardiac imaging; positron emission tomography.

IT IS WELL ESTABLISHED THAT myocardial ischemia is a powerful stimulus for vasodilatation of coronary resistive vessels. The vasodilator response is reported to be near maximal for ischemic times of up to 15–20 s, while no further changes are observed for longer ischemic times (16).

Experimental studies in animals have proven that intracoronary or intravenous infusion of adenosine achieves a degree of coronary vasodilatation comparable to that obtained after a period of ischemia of 15–20 s. In normal humans, intravenous infusion of 140 μg/kg adenosine produces an increase in myocardial blood flow (MBF) approximately fourfold above the resting value with no further increase at higher adenosine doses (12, 29).
evidenced by echocardiogram, and low clinical probability for CAD (6). Some of the healthy volunteers’ data have been published previously (15).

In addition, all study subjects were carefully instructed to refrain from intake of caffeine-containing beverages or food during the 24 h preceding the study. A screening test for caffeine was performed in a blood sample taken immediately before the positron emission tomography (PET) scan from each subject. Caffeine was not detectable in any of the blood samples.

**PET measurement of MBF.** MBF was measured using $^{15}$O-labeled water (H$_2^{15}$O) and an ECAT 931-08/12 15-slice PET scanner (CTI/Siemens, Knoxville, TX). H$_2^{15}$O (700–900 MBq) was injected as an intravenous bolus over 20 s at an infusion rate of 10 ml/min, and dynamic scanning was acquired over a period of 5.5 min (13, 14, 29). The sinograms obtained were corrected for attenuation and reconstructed on a MicroVax II computer (Digital Equipment, Marlboro, MA) employing dedicated array processors and standard reconstruction algorithms. On factor images, generated by iterative reconstruction, regions of interest were drawn within the left atrium and on left ventricular myocardium on consecutive image planes. These were projected onto the dynamic H$_2^{15}$O images to generate blood and tissue time activity curves, which were fitted to a single-tissue compartment tracer kinetic model to give values of MBF (ml·min$^{-1}$·g$^{-1}$) (12, 14, 26, 28–30).

Coronary flow reserve (CFR) was calculated as the ratio of MBF during adenosine to resting MBF. To account for the variability of coronary driving pressure, we calculated resting and minimal (i.e., during adenosine infusion) coronary resistance (mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$) as the ratio of mean systemic arterial pressure to MBF (10, 26).

**Study protocol.** Under baseline conditions, MBF was measured both at rest and during intravenous administration of adenosine (140 μg·kg$^{-1}$·min$^{-1}$), as previously reported (14, 28). Arterial blood pressure was recorded by an automatic cuff sphygmomanometer at 1-min intervals, and the ECG was monitored continuously throughout the procedure. A 12-lead ECG was recorded at baseline and every minute during adenosine administration. Thereafter, repeat measurements of MBF both at rest and during intravenous adenosine (140 μg·kg$^{-1}$·min$^{-1}$) were carried out following a 30-min intravenous infusion of 10 mg/kg l-NMMA (Cilagfa, Läufelfingen, Switzerland) dissolved in 50 ml of isotonic saline.

The study protocol was approved by the Research Ethics committees of Hammersmith Hospital. Radiation exposure was licensed by the UK Administration of Radioactive Substances Advisory Committee. All patients gave informed and written consent before the study.

**Statistical analysis.** Data are means ± SD. Statistical comparison of hemodynamic data, MBF, CFR, and coronary resistance during the different study conditions was carried out using analysis of variance for repeated measurements and post hoc Fisher’s protected least significant difference test. A P value <0.05 was considered significant.

**RESULTS**

**Baseline study.** The main hemodynamic parameters in normal volunteers and patients during the different study phases are reported in Table 1. No significant difference was detectable between the two groups except for a higher heart rate-systolic pressure product (RPP) in CAD patients at rest. In both groups, RPP increased significantly with adenosine.

In patients, resting MBF in territories subtended by a stenotic artery was not statistically different from MBF in normal volunteers, whereas MBF in remote myocardium subtended by a nonstenotic artery tended to be higher (Table 2 and Fig. 1). During adenosine infusion, the MBF increase in normal volunteers was greater than that observed in patients in territories subtended by a stenotic artery, whereas it was comparable to that in remote myocardium subtended by a nonstenotic artery (Table 2 and Fig. 1). CFR was significantly higher in volunteers than in CAD patients ($P < 0.01$ vs. remote myocardium and $P < 0.001$ vs. myocardium subtended by a stenotic artery) (see Table 2). Minimal coronary resistance (during intravenous adenosine) was 28.5 ± 9.9 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$ in normal volunteers, 64.1 ± 26.0 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$ in territories subtended by a stenotic artery ($P < 0.0005$ vs. healthy volunteers), and 32.2 ± 11.2 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$ in remote myocardium ($P = $ nonsignificant (NS) vs. healthy volunteers and $P < 0.005$ vs. stenotic territories).

**l-NMMA infusion.** Mean arterial pressure both at rest and during adenosine increased significantly after l-NMMA infusion, whereas corresponding heart rates were reduced (Table 1). Resting MBF was substantially unchanged in normal volunteers and patients both in territories subtended by a stenotic artery and in remote myocardium (Table 2 and Fig. 1). During adenosine infusion, MBF increased significantly compared with the respective baseline data both in normal volunteers and in patients. In the latter, there was a significant increase both in territories subtended by stenotic arteries and in remote myocardium. Similarly, CFR increased significantly in both groups (Table 2). Minimal coronary resistance decreased to 17.4 ± 3.1 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$ in normal volunteers ($P < 0.0005$ vs. baseline) and to 47.1 ± 18.8 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$ in territories subtended by a stenotic artery ($P < 0.05$ vs. baseline and $P < 0.0001$ vs. normal volunteers) and tended to decrease in remote myocardium (29.4 ± 15.3 mmHg·ml$^{-1}$·min$^{-1}$·g$^{-1}$, $P =$ NS vs. baseline and $P = 0.01$ vs. normal volunteers).

**DISCUSSION**

The main finding of the present study is that the systemic infusion of l-NMMA in patients with CAD significantly increases the MBF response to adenosine in territories subtended by stenotic (≥70% luminal diameter) coronary arteries. The results of the present study extend our previous observations in healthy volunteers and demonstrate that the maximum MBF

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**Table 1. Hemodynamics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th></th>
<th>Adenosine</th>
<th></th>
<th>l-NMMA</th>
<th></th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>HR</td>
<td>RPP</td>
<td>MAP</td>
<td>HR</td>
<td>RPP</td>
<td>MAP</td>
</tr>
<tr>
<td>1</td>
<td>86±9</td>
<td>59±9</td>
<td>6,714±1,239</td>
<td>90±9</td>
<td>93±15b</td>
<td>11,574±2,239b</td>
<td>95±7d</td>
</tr>
<tr>
<td>2</td>
<td>95±10</td>
<td>63±11</td>
<td>8,308±1,957</td>
<td>99±15</td>
<td>94±17b</td>
<td>13,306±3,572b</td>
<td>113±13d</td>
</tr>
<tr>
<td>P</td>
<td>ns</td>
<td>&lt;0.05</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>&lt;0.05</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are heart rate (HR; beats/min), mean arterial pressure (MAP; mmHg), and rate-pressure product (RPP; systolic blood pressure × HR) at baseline and after N$^\circ$-monomethyl-l-arginine (l-NMMA) infusion in controls (group 1) and coronary artery disease (CAD) patients (group 2). P values shown are for between-group comparison; ns, no significant difference between groups. *P < 0.05; **P < 0.005 vs. rest. +P < 0.05; ++P < 0.005 vs. respective baseline.
response to adenosine in humans is constrained by what could be a neurally mediated vasoconstriction of resistance vessels. This latter is relieved by systemic infusion of L-NMMA potentially via central neuronal NOS (nNOS) inhibition, as suggested by the lack of flow increase after L-NMMA observed in transplant recipients that was demonstrated in our group’s previous study (15).

The notion that the MBF response to adenosine and/or dipyridamole does not represent the maximum flow achievable in the coronary system has been previously demonstrated in both animals and humans. In open-chest anesthetized dogs, L’Abbate et al. (16) demonstrated that prolonged intracoronary infusion of adenosine provoked a biphasic flow response: at first, within 15–30 s, coronary flow increased to a level similar to that observed during reactive hyperemia after a 30-s coronary occlusion; subsequently, continuing the infusion led to further coronary vasodilatation that within 20 – 45 min from the start of the infusion reached a plateau at a value that was twice as much as that observed during reactive hyperemia and remained constant up to 2 h.

α-Adrenoceptor-mediated coronary vasoconstriction can compete with local metabolic vasodilation, as has been shown in a number of studies in dogs, limiting the coronary vascular response during sympathetic activation, e.g., exercise (4, 7, 9, 19), norepinephrine infusion, or carotid sinus reflex (18). The role of α-adrenoceptor-mediated coronary vasoconstriction has also been investigated during maximal vasodilation to overcome the confounding influence of autoregulation. In two different studies, during adenosine infusion, Vlahakes et al. (27) found higher flow after phentolamine and lower flow after phenylephrine, and Johannsen et al. (11) observed lower flow during cardiac sympathetic nerve stimulation and following infusion of phenylephrine or norepinephrine. However, although sympathetic discharge to the heart has been shown to result in both α-adrenergic constriction in the upstream vessels and β-adrenoceptor-mediated dilation in the coronary microcirculation, the latter has been found to be the dominant effect in pigs and dogs (24). This may not necessarily apply to humans. In fact, in a study in normal human volunteers in whom MBF was measured noninvasively by means of PET, Lorenzoni et al. (17) showed that the MBF response to dipyridamole was increased by 40% when the study was repeated during pharmacological blockade of α1-adrenoceptors.

Our data suggest that the reflex sympathetic activation elicited following the systemic administration of vasodilators such as adenosine and dipyridamole should result in a further fall in minimal coronary resistance. The latter, however, is blunted by an NO-modulated suppression of sympathetic outflow in the

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**Table 2. Myocardial blood flow and coronary flow reserve**

<table>
<thead>
<tr>
<th>Group</th>
<th>MBF, ml·min⁻¹·g⁻¹</th>
<th>CFR (Relative Values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline L-NMMA</td>
<td>Baseline L-NMMA</td>
</tr>
<tr>
<td>Controls</td>
<td>0.88±0.13</td>
<td>0.88±0.12</td>
</tr>
<tr>
<td></td>
<td>3.48±0.99</td>
<td>5.26±0.65</td>
</tr>
<tr>
<td></td>
<td>4.00±1.10</td>
<td>6.15±1.35</td>
</tr>
<tr>
<td>CAD Remote</td>
<td>1.06±0.22d</td>
<td>1.14±0.22d</td>
</tr>
<tr>
<td></td>
<td>3.27±0.96</td>
<td>4.34±1.59</td>
</tr>
<tr>
<td></td>
<td>3.20±1.23</td>
<td>3.92±1.62</td>
</tr>
<tr>
<td>CAD Stenosis</td>
<td>0.96±0.36</td>
<td>0.84±0.29</td>
</tr>
<tr>
<td></td>
<td>1.86±0.94e</td>
<td>2.69±1.24</td>
</tr>
<tr>
<td></td>
<td>2.06±1.13e</td>
<td>3.21±1.07</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are myocardial blood flow (MBF) at baseline and after adenosine infusion and coronary flow reserve (CFR) at baseline and after L-NMMA infusion in controls and in territories subtended by a stenotic artery and in remote myocardium in CAD patients. P values shown are for within-group comparison (CAD). *P < 0.05; **P < 0.01; ***P < 0.0001 vs. baseline. dP < 0.05; eP < 0.005 vs. controls.
central nervous system, where neuronal NO modulates neurotransmitter release (20). This is in line with our previous study, which showed that an increase in the standard adenosine infusion rate of 140 μg·kg⁻¹·min⁻¹ (12) does not further decrease coronary resistance, whereas systemic confusion of adenosine with l-NMMA further decreased coronary resistance in healthy volunteers (15).

In the present study, the increase in MBF was significant in both ischemic and remote territories of CAD patients. Nevertheless, CFR remained significantly lower in remote segments than in controls after l-NMMA. This is in line with previous observations in CAD patients reporting an impaired hyperemic response in remote territories supplied by an angiographically normal coronary artery (5, 23).

It remains controversial whether inhibition of nNOS elicits sympathoexcitatory effects (21) or not (8), since it may exert opposing effects at different sites (22). The present results in CAD patients indicate 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.

Limitations of the study. Although our observations support the above suggestion that neurally mediated vasoconstriction is relieved by systemic NOS inhibition with l-NMMA, this must remain a hypothesis. Similarly, we cannot comment on a possible involvement of a parasympathetic component. To provide final proof, a future study would need to demonstrate, for example, that an α-adrenergic blockade causes augmentation of coronary flow rates during adenosine similar to that produced by l-NMMA [which has been reported by Lorenzoni et al. (17) as mentioned above], and second, that after l-NMMA administration, α-adrenergic blockade would not further increase MBF response to adenosine. This, however, was beyond the scope of the present study.

An additional limitation may be that CAD patients were older than controls, and this difference may potentially have hampered the comparability of the two groups’ CFR, since the latter has been shown to decrease after the age of 60 yr (25), although mainly because of an increase in basal flow (3). By contrast, maximal hyperemic response decreases only after the age of 70 yr (2), which would be irrelevant to our study since none of our patients was older than 68 yr. Furthermore, the experimental design using every subject as his own control before and after l-NMMA administration and comparing stenotic versus remote segments within each subject further strengthens our results.

l-NMMA induced a significant increase in mean arterial pressure, which could theoretically have contributed to the increase in hyperemic response after l-NMMA. This, however, was been ruled out in our group’s previous study, in which phenylephrine was used to increase blood pressure but did not affect hyperemic response and CFR (4).

In conclusion, the present study provides evidence that adenosine-induced hyperemic flow response in CAD patients is constrained, potentially by neurally mediated vasoconstriction, and that this can be relieved by systemic NOS inhibition with l-NMMA. Further studies are needed to ascertain whether the findings of this investigation offer the possibility of devising some new form of treatment to improve myocardial perfusion in patients with chronic CAD.

REFERENCES


GRANTS

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