Cardiovascular Effects of Systemic Nitric Oxide Synthase Inhibition With Asymmetrical Dimethylarginine in Humans

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Background—Increased blood concentrations of the endogenous nitric oxide synthase (NOS) inhibitor asymmetrical dimethylarginine (ADMA) have been linked to excess cardiovascular morbidity and mortality and to progression of renal disease. We evaluated systemic cardiovascular effects of ADMA infusion in healthy subjects using invasive techniques, ie, right heart catheter and inulin/para-aminohippurate clearance.

Methods and Results—Plasma ADMA concentrations encountered in patients with cardiovascular diseases, ie, between 2 and 10 μmol/L, caused a significant (P<0.05) decrease in concentrations of plasma cGMP, the main second messenger of NO. In addition, cardiac output was significantly lower (5.3±0.6 versus 5.8±0.6 L/min; P<0.05 versus baseline), and systemic vascular resistance was significantly higher (1403±123 versus 1221±100 dyn · s · cm⁻⁵; P<0.05 versus baseline). The infusion of 0.25 mg ADMA · kg⁻¹ · min⁻¹ or 3 μg N⁵-nitro-L-arginine methyl ester · kg⁻¹ · min⁻¹, a potent synthetic NOS inhibitor with long action, resulted in a comparable decrease in effective renal plasma flow (from 670±40 to 596±29 mL · min⁻¹; P<0.05) and an increase in renovascular resistance (from 79±5 to 90±7 mm Hg · mL⁻¹ · min⁻¹; P<0.05). Moreover, administration of ADMA caused significant sodium retention and blood pressure increase (both P<0.05). The observed effects of ADMA in the systemic circulation were sustained corresponding to a mean plasma half-life of 23.5±6.8 minutes, calculated from plasma ADMA decay curves in healthy subjects.

Conclusions—Systemic ADMA infusion is responsible for a short-term, modest decrease in cardiac output with comparable decrease in effective renal plasma flow while increasing systemic vascular resistance and blood pressure in a dose-related manner. (Circulation. 2004;109:172-177.)

Key Words: nitric oxide synthase • vasculature • asymmetrical dimethylarginine

Endothelium-derived nitric oxide (NO) plays a critical role in the regulation of endothelial cell function. It exerts its cardiovascular effects primarily via stimulation of soluble guanylate cyclase to produce cGMP.¹,² There are abundant experimental data that endothelial dysfunction caused by reduced availability of NO is an early step in the course of atherosclerotic vascular disease. Evidence has accumulated that inhibition of NO synthesis by endogenous inhibitors of the NO synthase (NOS) may be causally involved in this process.³ In 1992, Vallance et al⁴ first reported markedly elevated plasma levels of the NOS inhibitor asymmetrical dimethylarginine (ADMA) in patients with end-stage renal disease. In the following decade, a number of studies in renal and in nonrenal patients have been published in which a strong correlation between ADMA blood levels and increased cardiovascular morbidity and mortality was documented.⁵–¹⁰ Hence, ADMA not only is a biochemical marker of atherosclerosis but also is thought to play a causal role in its genesis.³,¹¹ Moreover, recent experimental studies have revealed that reduced bioavailability of NO plays a critical role in the progression of renal disease,¹²,¹³ and increased plasma ADMA levels may contribute to this process.¹⁴ Evidence for a biological action of ADMA is limited primarily to in vitro studies and studies in animals.¹⁴–²⁰ Controlled trials examining the effects of ADMA on different vascular beds in humans have not yet been reported. We infused ADMA intravenously in escalating doses to healthy subjects and assessed corresponding plasma concentrations and dose-response effects on NO production and renal hemodynamics. We have chosen this experimental setting because the human (postglomerular) renal circulation is very sensitive to NOS inhibition and can be easily assessed with accurate invasive clearance techniques.²¹,²² In a second study, we compared the renal effects of ADMA with those of N⁵-nitro-
l-arginine methyl ester (L-NAME), a potent synthetic NOS inhibitor for clinical use with long-lasting action.\cite{22,23} Finally, we assessed the effect of ADMA on systemic cardiovascular parameters, eg, cardiac output and systemic vascular resistance, by placing a right heart catheter in healthy volunteers.

**Methods**

**Subjects and Protocols**

All study protocols were approved by the Ethics Committee of the Hannover Medical School. Written informed consent was given by all participants, who were healthy nonsmoking male volunteers. During the studies, all participants adhered to an isocaloric standardized diet.

In a first series of experiments, we assessed safety, plasma ADMA concentrations achieved, and the dose-response effects on NO production and renal hemodynamics of a systemic ADMA infusion (N\textsuperscript{G},N\textsuperscript{G}-ADMA, Sigma Chemicals) in 6 healthy subjects. In each volunteer, the following doses were administered intravenously on 7 separate study days: 0 (placebo infusion), 0.0125, 0.025, 0.0375, 0.075, 0.15, and 0.25 mg ADMA · kg\textsuperscript{-1} · min\textsuperscript{-1}. ADMA was prepared by sterile filtration and dissolved in 50 mL of a 0.9% NaCl solution. The lowest ADMA dose was chosen on the basis of results of animal studies.\cite{23} The infusion of 0.25 mg · kg\textsuperscript{-1} · min\textsuperscript{-1} was the highest dose approved by the Ethics Committee. Participants were admitted to our metabolic ward at 8 PM on the day before the experiments to minimize environmental stress. The next morning, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were examined with the subjects in the supine position by use of steady-state inulin and para-aminohippurate (PAH) infusion techniques as described in detail elsewhere.\cite{22} After an appropriate equilibration period, blood samples for measurements of baseline GFR and ERPF were taken at regular intervals during a steady-state period of 50 minutes. Thereafter, ADMA was infused for 40 minutes, and renal hemodynamics were assessed in this period and after discontinuation of the infusion for another 50 minutes. Mean arterial blood pressure (MAP) was monitored noninvasively at regular intervals throughout with Dinamap (Critikon Co). Blood samples for measurements of plasma ADMA and cGMP concentrations were taken at the start (minute 50) and the end (minute 90) of the infusion period and immediately centrifuged at 1.500g at 4°C for 10 minutes, and the supernatants stored in aliquots at −80°C until further use.

In addition to the above protocol, blood samples for measurement of plasma ADMA levels were taken at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, and 60 minutes after discontinuation of infusion of 0.025 mg ADMA · kg\textsuperscript{-1} · min\textsuperscript{-1} (n=6). We calculated the disappearance plasma half-life of ADMA from the plasma concentration decay profile in each subject individually. We fitted the plasma ADMA concentration time series to a decay model that takes into consideration the admixture of a persistent simultaneous low level of endogenous ADMA production, given by the equation $C_{\text{ADMA}} = C_0 e^{-k t} + b$, where $k$ is ln2/half-life and b is the postdecay baseline ADMA concentration.

Using a double-blind placebo-controlled crossover study design, we allocated 11 healthy men in random order to infusion of either placebo, 0.25 mg ADMA · kg\textsuperscript{-1} · min\textsuperscript{-1}, or 3 µg L-NAME · kg\textsuperscript{-1} · min\textsuperscript{-1}. These infusions were administered on separate days, and GFR, ERPF, and MAP were assessed as described above. The synthetic NOS inhibitor L-NAME was chosen because of its long duration of action. For this reason, hemodynamic parameters were assessed for a total of 120 minutes after discontinuation of the infusions. The infusion rate of L-NAME was adopted from studies in healthy volunteers\cite{23} to achieve an effect on ERPF comparable to that observed with 0.25 mg ADMA · kg\textsuperscript{-1} · min\textsuperscript{-1} (Table 1). Blood samples for measurements of plasma ADMA and cGMP concentrations were drawn before (minute 50) and immediately after the infusion period (minute 90) and at the end of the postinfusion period (minute 210). In addition, we obtained urine samples from all subjects after the postinfusion period for assessment of urinary sodium excretion.

Finally, in a third series of experiments, we infused 0.10 mg ADMA · kg\textsuperscript{-1} · min\textsuperscript{-1} to 7 volunteers and invasively assessed cardiac output, systemic vascular resistance, and heart rate (ECG monitoring). For this purpose, a right heart catheter was placed under local anesthesia, and measurements were performed as described previously.\cite{23} After an appropriate equilibration period to minimize stress after the catheter placement, systemic hemo-

**TABLE 1. Effect of Escalating Doses of a Systemic ADMA Infusion on Plasma ADMA and cGMP Levels, Renal Hemodynamics, and Blood Pressure in 6 Healthy Subjects**

<table>
<thead>
<tr>
<th>ADMA Dose, mg · kg\textsuperscript{-1} · min\textsuperscript{-1}</th>
<th>0</th>
<th>0.0125</th>
<th>0.025</th>
<th>0.0375</th>
<th>0.075</th>
<th>0.15</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ADMA concentration, µmol/L</td>
<td>Pre 0.94±0.26</td>
<td>1.02±0.26</td>
<td>0.96±0.28</td>
<td>1.00±0.30</td>
<td>1.13±0.25</td>
<td>1.10±0.22</td>
<td>1.04±0.22</td>
</tr>
<tr>
<td>Post 1.03±0.28</td>
<td>4.17±0.99</td>
<td>8.13±2.08</td>
<td>11.84±2.53</td>
<td>15.66±4.29</td>
<td>32.92±6.68</td>
<td>42.13±12.74</td>
<td></td>
</tr>
<tr>
<td>Plasma cGMP concentration, pmol/L</td>
<td>Pre 6.1±1.7</td>
<td>5.9±1.8</td>
<td>5.8±1.2</td>
<td>6.1±1.2</td>
<td>6.0±1.2</td>
<td>6.0±1.5</td>
<td>6.2±1.2</td>
</tr>
<tr>
<td>Post 6.2±1.6</td>
<td>5.1±1.8*</td>
<td>4.8±0.9*</td>
<td>4.8±1.3*</td>
<td>4.5±0.9*</td>
<td>4.3±1.2*</td>
<td>4.0±0.5*</td>
<td></td>
</tr>
<tr>
<td>Glomerular filtration rate, mL · min\textsuperscript{-1} · 1.73 m\textsuperscript{-2}</td>
<td>Pre 120±6</td>
<td>120±10</td>
<td>122±8</td>
<td>122±6</td>
<td>121±7</td>
<td>121±11</td>
<td>122±10</td>
</tr>
<tr>
<td>Post 121±7</td>
<td>122±10</td>
<td>125±7</td>
<td>123±6</td>
<td>122±8</td>
<td>122±11</td>
<td>123±11</td>
<td></td>
</tr>
<tr>
<td>Effective renal plasma flow, mL · min\textsuperscript{-1} · 1.73 m\textsuperscript{-2}</td>
<td>Pre 664±31</td>
<td>662±37</td>
<td>662±43</td>
<td>669±30</td>
<td>664±40</td>
<td>665±28</td>
<td>662±23</td>
</tr>
<tr>
<td>Post 668±32</td>
<td>667±29</td>
<td>658±38</td>
<td>648±27</td>
<td>621±42*</td>
<td>603±26*</td>
<td>589±16*</td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>Pre 86±5</td>
<td>86±4</td>
<td>84±6</td>
<td>85±8</td>
<td>86±4</td>
<td>86±5</td>
<td>85±4</td>
</tr>
<tr>
<td>Post 86±5</td>
<td>84±6</td>
<td>84±5</td>
<td>86±8</td>
<td>85±9</td>
<td>87±5</td>
<td>89±3</td>
<td></td>
</tr>
</tbody>
</table>

All infusions were administered on separate days. Pre indicates preinfusion; post, postinfusion.

*P<0.05 preinfusion (baseline) vs postinfusion data.
dynamics were assessed at regular time points during a placebo infusion period (50 minutes), followed by the ADMA infusion (40 minutes), and for another 120 minutes after discontinuation of the ADMA infusion. The mean of 4 measurements at each time point was taken into analysis. Blood samples for measurement of ADMA levels were taken before (minute 50) and after (minute 90) the infusion period and at the end of the postinfusion period (minute 210). The infusion rate was chosen on the basis of the results from the dose-response study.

Measurements and Calculations
Inulin plasma concentration was measured enzymatically with inulinase and that of PAH photometrically, and inulin and PAH clearances were calculated as described previously. Filtration fraction was calculated as the ratio between inulin and PAH clearance, and renovascular resistance (RVR) was calculated by the equation 

\[
RVR = \frac{\text{MAP} - 12 \times 723/\text{ERPF}}{\text{MAP} - 12 - 723/\text{ERPF}}
\]

Plasma ADMA levels were determined by high-performance liquid chromatography with precolumn derivatization with o-phthalaldehyde as described previously. The coefficients of variation of this method is 5.2% within assay and 5.5% between assay; the detection limit of the assay is 0.1 \(\mu\text{mol/L}\). Plasma cGMP was measured by ELISA (R&D Systems).

Statistical Analysis
The primary efficacy parameters in the dose-finding experiments were plasma cGMP concentration and ERPF. We compared preinfusion (baseline) and postinfusion values using a Student paired \(t\) test (SPSS statistical package). All other data were analyzed descriptively. In the comparative trial with placebo, ADMA, and L-NAME infusion, we compared preinfusion (baseline) and postinfusion (minute 210) data of the 3 groups by ANOVA. If this procedure revealed significant differences, we used a paired \(t\) test to compare means between different infusion protocols; Bonferroni correction was applied. In addition, we compared intraindividual preinfusion (baseline) and postinfusion (minute 210) values using a paired \(t\) test. The latter test was also used to compare data on systemic cardiovascular parameters obtained during the right heart catheter experiments. The significance level was set at \(P<0.05\). Data in text and in tables are presented as mean±SD, whereas data in figures are presented as mean±SEM.

Results
The effect of increasing systemic ADMA blood levels on NO production, GFR, ERPF, and blood pressure are summarized in Table 1. Acute increase of plasma ADMA levels within the pathophysiologically relevant range, ie, between 2 and 10 \(\mu\text{mol/L}\), were achieved with infusion rates of 0.0125 and 0.025 mg ADMA \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\). With these ADMA doses, we observed a significant decrease in plasma cGMP concentrations. A significant effect on ERPF was documented with infusion rates of 0.075 mg ADMA \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) and above, whereas GFR remained unaffected (Table 1). Mean plasma ADMA half-life calculated from plasma concentration decay profiles of 6 healthy subjects was 23.5±6.8 minutes, and the individual half-lives were 22.8, 25.4, 20.4, 35.9, 19.9, and 16.4 minutes.

Infusion of 3.0 \(\mu\text{g}\) L-NAME \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) and 0.25 mg ADMA \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) caused a significant decrease in ERPF and a significant increase in filtration fraction and RVR (Figure 1). The effect of ADMA on ERPF and RVR was observed immediately after the start of the infusion, whereas the effect of L-NAME with some delay. The effect on renal perfusion was sustained with administration of both L-NAME and ADMA, lasting for at least 2 hours.

Figure 1. Effect of placebo infusion (O), infusion of 3.0 \(\mu\text{g}\) L-NAME \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) (□), and infusion of 0.25 mg ADMA \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) (■) on renal hemodynamics in 11 healthy volunteers. A, GFR; B, ERPF; C, filtration fraction (FF); D, RVR. \(^*P<0.05\), preinfusion (baseline) vs postinfusion (minute 210) data; \#P<0.05, ADMA vs placebo infusion; \$P<0.05, L-NAME vs placebo infusion.
after the end of infusions (Figure 1). Moreover, infusion of both L-NAME and ADMA caused a small but significant increase in MAP (Figure 2). Plasma ADMA and cGMP levels are shown in Table 2. Two hours after discontinuation of the infusion, mean ADMA blood concentration was $<10$ μmol/L. In contrast, plasma cGMP concentration decreased significantly with administration of both NOS inhibitors and was still $30\%$ below baseline values 2 hours after the infusions stopped. The changes in NO production and renal hemodynamics with ADMA and L-NAME infusion were accompanied by a significant decrease in renal sodium excretion. Urinary sodium excretion was lower with infusion of ADMA ($127\pm26$ μmol/min) and L-NAME ($123\pm28$ μmol/min) than with placebo infusion ($153\pm23$ μmol/min; $P<0.05$ versus both NOS inhibitors).

Infusion of ADMA to 7 healthy subjects caused a significant and sustained decrease in cardiac output (Figure 3A) and a significant increase in systemic vascular resistance (Figure 3B). Like the response of the renal circulation, we observed an immediate effect of ADMA on systemic cardiovascular parameters, and the action of ADMA lasted until the end of the postinfusion period (minute 210). In addition, we observed a significant ($P<0.05$) decrease in heart rate during ADMA infusion from $58\pm7$ to $54\pm6$ bpm; at the end of the postinfusion period, mean heart rate was $56\pm8$ bpm. Mean plasma ADMA concentration increased from $0.95\pm0.27$ (baseline) to $22.95\pm4.91$ μmol/L at the end of the infusion period (minute 90). It was $5.31\pm1.43$ μmol/L at the end of the postinfusion period (minute 210), ie, within a pathophysiological relevant range.

**Table 2. Effect of Placebo, L-NAME (3.0 μg · kg$^{-1}$ · min$^{-1}$), and ADMA (0.25 mg · kg$^{-1}$ · min$^{-1}$) Infusion on Plasma ADMA and cGMP Concentrations in 11 Healthy Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>L-NAME</th>
<th>ADMA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma ADMA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preinfusion (baseline)</td>
<td>$1.15\pm0.50$</td>
<td>$1.13\pm0.46$</td>
<td>$1.20\pm0.48$</td>
</tr>
<tr>
<td>Postinfusion (min 90)</td>
<td>$1.20\pm0.50$</td>
<td>$1.25\pm0.43$</td>
<td>$47.57\pm12.65^{\dagger\ddagger}$</td>
</tr>
<tr>
<td>2 h postinfusion (min 210)</td>
<td>$1.21\pm0.48$</td>
<td>$1.16\pm0.38$</td>
<td>$8.57\pm2.65^{\dagger\ddagger}$</td>
</tr>
<tr>
<td><strong>Plasma cGMP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preinfusion (baseline)</td>
<td>$5.8\pm1.2$</td>
<td>$5.9\pm1.1$</td>
<td>$6.1\pm1.3$</td>
</tr>
<tr>
<td>Postinfusion (min 90)</td>
<td>$5.6\pm1.5$</td>
<td>$4.9\pm1.5^{$\ddagger}$</td>
<td>$4.6\pm1.0^{\dagger\ddagger}$</td>
</tr>
<tr>
<td>2 hours postinfusion (min 210)</td>
<td>$5.9\pm1.2$</td>
<td>$3.9\pm0.94^{$}$</td>
<td>$4.0\pm0.7^{\dagger\ddagger}$</td>
</tr>
</tbody>
</table>

$^{\dagger}P<0.05$, ADMA vs placebo infusion.

$^{\ddagger}P<0.05$, ADMA vs L-NAME infusion.

$^{\$}P<0.05$, preinfusion (baseline) vs postinfusion data.

$^{\$\ddagger}P<0.05$, L-NAME vs placebo infusion.
Discussion

Our results, obtained in a series of controlled clinical experiments with invasive assessment of cardiovascular parameters, document for the first time that systemic administration of the (endogenous) NOS inhibitor ADMA has definite effects on cardiovascular and renal function in healthy subjects. We observed these effects after infusion of doses that acutely yielded plasma ADMA levels above those encountered in patients with renal and/or cardiovascular disease.\textsuperscript{4, 7, 9, 28, 29} but administration of even smaller doses caused a significant decrease in plasma cGMP concentrations, ie, the main second messenger of NO in the cardiovascular system. Moreover, after discontinuation of the infusion, plasma ADMA levels returned to the pathophysiologically relevant range, ie, to <10 μmol/L. At this concentration, the systemic and renal hemodynamic effects of ADMA in healthy subjects were still clearly measurable, however. It is therefore conceivable that ADMA causes sustained changes in vascular function through an intracellular action in endothelial cells at blood concentrations found in patients with cardiovascular pathological conditions.

Indirect evidence for the above assumption comes from a recent study in renal patients, in whom plasma ADMA levels were increased ~4 times compared with healthy control levels. Ex vivo, their blood markedly inhibited NO production in cultured endothelial cells.\textsuperscript{30} With the lowest infusion rate chosen, plasma ADMA levels increased acutely ~4-fold in healthy subjects and caused a significant reduction in NO production. Our results are therefore in line with evidence accumulated from experimental studies that indicate that ADMA is a potent endogenous NOS inhibitor.\textsuperscript{3, 12–14} As a consequence, chronically elevated plasma ADMA levels may be of definite relevance in human vascular pathology. A rapidly growing number of published clinical studies documenting a strong correlation between increased ADMA levels and cardiovascular morbidity and mortality in different populations support this hypothesis.\textsuperscript{5–8, 10, 29, 31}

We have chosen L-NAME for direct comparison with ADMA with respect to NOS inhibition because of its long-lasting action. The response of ERPF and RVR in our healthy subjects to administration of 3 μg L-NAME·kg\textsuperscript{-1}·min\textsuperscript{-1} was similar to that found in previous studies.\textsuperscript{22, 22, 24} With infusion of either L-NAME or ADMA, we observed a sustained decrease in ERPF and an increase in RVR and MAP. It is therefore conceivable that higher ADMA doses cause a larger increase in blood pressure, similar to that shown for L-NAME.\textsuperscript{21, 23, 24} In addition, our finding of significant renal vasoconstriction with ADMA infusion yielding acute plasma levels in the supraphysiological range is reminiscent of the action of angiotensin II. The latter exerts renal vasoconstrictor effects at blood levels that are above those found in patients with hypertension and/or cardiovascular disease.\textsuperscript{32} Nevertheless, angiotensin II has a number of effects promoting atherogenesis, possibly because of high (local) tissue concentrations. The latter may be true for ADMA as well, and chronically elevated ADMA blood and/or tissue levels may cause vascular injury at the endothelial cell level.

Of particular interest in this respect is the time course of the action of ADMA. Like L-NAME, ADMA has a long duration of action, contrasting with the short-lived action of NO. This is in accordance with the relatively long ADMA plasma half-life of >20 minutes calculated from plasma decay curves in healthy subjects. The plasma half-life could theoretically be even longer in conditions in which the main pathway of ADMA degradation by the enzyme dimethylarginine dimethylaminohydrolase is dysregulated, such as in hypercholesterolemia and diabetes mellitus.\textsuperscript{33, 34} In addition to its long-lasting action, the effect of ADMA was already seen within the infusion period, pointing to immediate NOS inhibition. In this respect, ADMA differs from L-NAME, which is a prodrug and has first to be metabolized to its active form, ie, N\textsuperscript{\textsuperscript{-}}-nitro-L-arginine.\textsuperscript{35}

In conclusion, systemic ADMA infusion is responsible for a short-term, modest decrease in cardiac output with a comparable decrease in ERPF while increasing systemic vascular resistance and blood pressure in a dose-related manner.

Acknowledgments

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References


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