Asymmetric Dimethylarginine Causes Hypertension and Cardiac Dysfunction in Humans and Is Actively Metabolized by Dimethylarginine Dimethylaminohydrolase

Vinod Achan, Michael Broadhead, Mohammed Malaki, Guy Whitley, James Leiper, Raymond MacAllister, Patrick Vallance

Objective—Plasma levels of an endogenous nitric oxide (NO) synthase inhibitor, asymmetric dimethylarginine (ADMA), are elevated in chronic renal failure, hypertension, and chronic heart failure. In patients with renal failure, plasma ADMA levels are an independent correlate of left ventricular ejection fraction. However, the cardiovascular effects of a systemic increase in ADMA in humans are not known.

Methods and Results—In a randomized, double-blind, placebo-controlled study in 12 healthy male volunteers, we compared the effects of intravenous low-dose ADMA and placebo on heart rate, blood pressure, cardiac output, and systemic vascular resistance at rest and during exercise. We also tested the hypothesis that ADMA is metabolized in humans in vivo by dimethylarginine dimethylaminohydrolase (DDAH) enzymes. Low-dose ADMA reduced heart rate by $9.2\pm1.4\%$ from $58.9\pm2.0$ bpm ($P<0.001$) and cardiac output by $14.8\pm1.2\%$ from $4.4\pm0.3$ L/min ($P<0.001$). ADMA also increased mean blood pressure by $6.0\pm1.2\%$ from $88.6\pm3.4$ mm Hg ($P<0.005$) and SVR by $23.7\pm2.1\%$ from $1639.0\pm91.6$ dyne $\cdot$ s $\cdot$ cm$^{-5}$ ($P<0.001$). Handgrip exercise increased cardiac output in control subjects by $96.8\pm23.3\%$, but in subjects given ADMA, cardiac output increased by only $35.3\pm10.6\%$ ($P<0.05$). DDAHs metabolize ADMA to citrulline and dimethylamine. Urinary dimethylamine to creatinine ratios significantly increased from $1.26\pm0.32$ to $2.73\pm0.59$ after ADMA injection ($P<0.01$). We estimate that humans generate approximately $300\,\mu$mol of ADMA per day, of which approximately $250\,\mu$mol is metabolized by DDAHs.

Conclusions—This study defines the cardiovascular effects of a systemic increase in ADMA in humans. These are similar to changes seen in diseases associated with ADMA accumulation. Finally, our data also indicate that ADMA is metabolized by DDAHs extensively in humans in vivo. (Arterioscler Thromb Vasc Biol. 2003;23:1455-1459.)

Key Words: asymmetric methylarginine • dimethylarginine dimethylaminohydrolase • nitric oxide • hypertension • cardiac output

Nitric oxide (NO) plays an important role in the regulation of cardiovascular function, and asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthesis, has been implicated in the impairment of NO generation in a variety of cardiovascular diseases. The finding that plasma ADMA levels are elevated in chronic renal failure led to speculation that ADMA may in part be responsible for increased cardiovascular risk and hypertension in these patients. Subsequent studies have shown strong associations between raised ADMA levels and cardiovascular risk factors, endothelial dysfunction, hypertension, atherosclerosis, and cardiovascular mortality. ADMA levels are also significantly raised in patients with chronic heart failure, rats with heart failure induced by coronary artery ligation, and dogs with heart failure induced by rapid pacing. A recent analysis of a large group of patients with end-stage renal failure demonstrated an inverse relationship between ADMA levels and left ventricular (LV) ejection fraction; in a multivariate analysis that included LV end diastolic volume and heart rate, ADMA was shown to be an independent and strong predictor of LV ejection fraction. These findings raise the possibility that a causal relationship between ADMA and LV dysfunction may exist.

The aim of the present study was to test the hypothesis that administration of low-dose ADMA to healthy volunteers would produce cardiovascular changes similar to those seen in diseases associated with ADMA accumulation. We also set out to test the hypothesis that ADMA is extensively metabolized by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes in humans in vivo.

Methods
The protocol was approved by the University College London Research Ethics Committee. Twelve healthy, normotensive men...
(aged 23 to 42 years) gave informed and signed consent to participate in the study. All studies were performed in a temperature-controlled laboratory (24 to 26°C).

Drugs
ADMA was obtained from Paragon Biochemical, checked for purity by high-performance liquid chromatography, and proven to be endotoxin free (Biowhittaker). ADMA was dissolved in sterile physiological saline, stored at −80°C, and sterile filtered immediately before use (0.22 mm Millex-GP, Millipore). Placebo (sterile physiological saline) was also stored at −80°C.

Hemodynamic Measurements
All studies were done with subjects lying supine and with continuous electrocardiographic monitoring. Systolic and diastolic blood pressures were measured every 5 minutes using a semiautomated sphygmomanometer placed over the right arm. Cardiac output (CO) measurements were made noninvasively by bioimpedance cardiology (Physio Flow, Manatec Biomedical) as described previously. Systemic vascular resistance (SVR; dyne ∙ s ∙ cm−5) was calculated according to the standard formula, SVR=80(MAP−CVP)/CO, where MAP is mean arterial pressure and CVP is central venous pressure. CO and heart rate were measured as the average value of 3 consecutive beats every 10 seconds. For readings at rest, the mean of 10 such readings was taken. During handgrip exercise, the highest value recorded during the 1-minute period was used for analysis.

Protocol
The study design was randomized, double-blind, and placebo-controlled. Volunteers were randomly allocated to receive an intravenous injection of either low-dose ADMA (3 mg/kg up to a maximum of 250 mg) or placebo (sterile physiological saline). Volunteers were asked to avoid seafood in their diet for 24 hours before and during the study to minimize dietary dimethylamine intake. An 18-gauge intravenous cannula was inserted into a deep antecubital vein in both arms. A baseline blood sample was collected from the left arm before a 30-minute phase of baseline hemodynamic measurements in the supine position. Subjects then received an injection of ADMA or placebo into the right arm. An additional blood sample was taken 30 minutes after injection. Hemodynamic measurements were continued for 60 minutes after injection. At the end of this period, subjects performed a handgrip exercise protocol for 1 minute using the right hand (40 compressions to a pressure of 200 mm Hg). Urine samples were collected at baseline and 2 hours after injection. All blood samples were centrifuged and separated immediately. Samples were stored at −80°C until further analysis.

Biochemical Measurements
Plasma methylarginine concentrations were determined by high-performance liquid chromatography as previously described. Dimethylamine levels in the urine (μmol/L) were determined by the method of Beal and Bryan. Urinary creatinine (Cr) levels (mg/dL) were measured using a modification of the method of Slot.

Statistics
Data are expressed as mean±SEM. The overall statistical significance of ADMA versus placebo data was tested using repeated-measures ANOVA; if positive, data from individual time points were tested additionally by the Student’s paired t test. Other comparisons between 2 groups were also evaluated using the Student’s paired t test. P<0.05 was considered statistically significant.

Results
No differences in baseline weight, heart rate, cardiac output, mean blood pressure, and systemic vascular resistance were found between the 2 study groups. Of all the parameters studied, heart rate changed first such that the effect was evident within the first minute in subjects given ADMA (Figure 1A). Intravenous injection of ADMA reduced heart rate by 9.2±1.4% from 58.9±2.0 bpm (after 5 minutes; P<0.001) and cardiac output by 14.8±1.2% from 4.4±0.3 L/min (after 10 minutes; P<0.001). ADMA increased mean blood pressure by 6.0±1.2% from 88.6±3.4 mm Hg (after 10 minutes; P<0.005) and SVR by 23.7±2.1% from 1639.0±91.6 dyne ∙ s ∙ cm−5 (after 10 minutes; P<0.001) (Figure 1B). Changes in heart rate and cardiac output returned to baseline during the 60 minutes after injection. Other parameters approached, but did not reach, baseline values by the end of the study. No significant changes were seen in subjects receiving placebo. Handgrip exercise increased car-
Cardiac output in control subjects by a maximum of 96.8±23.3%. In contrast, subjects given ADMA only increased their cardiac output by 35.3±10.6% during exercise ($P<0.05$ compared with placebo) (Figure 2). Blood pressure was recorded at the beginning and end of exercise and did not change. Heart rate increased in both groups, and there was no statistical difference between the groups. No analysis of SVR was undertaken because of the lack of blood pressure measurements during exercise.

In subjects that received ADMA, the plasma ADMA concentration increased to 2.6±0.3 μmol/L at 30 minutes after the injection ($P<0.001$ compared with baseline). Symmetric dimethylarginine (SDMA) levels did not change significantly (data not shown).

The urinary dimethylamine (μmol/L) to Cr (mg/dL) ratio was unaltered after placebo but increased from 1.26±0.32 to 2.73±0.59 after ADMA injection ($P<0.05$) (Figure 3). Assuming a normal urinary Cr excretion in men of 1.1 to 2.8 g/d, normal baseline urinary dimethylamine excretion was estimated at 260±30 μmol/d.

**Discussion**

This study shows that a systemic increase in the endogenous NO synthase (NOS) inhibitor ADMA produces adverse cardiovascular effects in humans. In a randomized, double-blind, placebo-controlled study in healthy volunteers, an intravenous injection of ADMA significantly reduced heart rate and cardiac output and increased blood pressure and systemic vascular resistance. Furthermore, subjects receiving ADMA showed an impaired cardiac output response to upper limb exercise. Injection of ADMA also increased urinary dimethylamine excretion, demonstrating that the ADMA/DDAH pathway is active in humans. Together, these results support a causal role relationship between raised ADMA levels and cardiovascular dysfunction and suggest that the metabolism of ADMA by DDAH is likely to be an important regulatory mechanism in the human cardiovascular system.

ADMA is a naturally occurring methylarginine that inhibits all 3 isoforms of NOS. ADMA has been shown to inhibit endothelial NOS (eNOS) in vitro, in animals, and in the human forearm arterial bed. However, the systemic effects of ADMA have not been studied in humans previously. We used a bolus dose of ADMA to assess the speed of onset of...
response and to determine its duration in the face of ongoing ADMA metabolism. The results of this randomized, double-blind, controlled study show clearly that ADMA produces major hemodynamic changes at rest that are similar but not identical to those previously reported for N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA).\textsuperscript{15,16} ADMA and L-NMMA are structurally similar and have similar effects on isolated eNOS.\textsuperscript{3} Nonetheless, differences between ADMA and L-NMMA effects have been reported,\textsuperscript{17} and it is important to define the systemic effects of ADMA in humans in vivo. ADMA produced a large increase in systemic vascular resistance in association with a fall in cardiac output, and as a result the overall rise in blood pressure was relatively modest. It is not known whether the change in cardiac output was secondary to the change in blood pressure or whether it represents a direct effect of NOS inhibition on cardiac function.\textsuperscript{18} The effect was most marked when cardiac output increased in response to exercise; indeed, ADMA infusion markedly blunted this physiological response. ADMA levels correlate with LV dysfunction and reduced ejection fraction in patients with renal failure\textsuperscript{11} and are also elevated in patients with heart failure.\textsuperscript{8} Our results suggest that ADMA may have a role in the pathophysiology of certain types of cardiac dysfunction.

Interestingly, the most rapid change seen in response to ADMA was a change in heart rate. A significant fall in heart rate was seen almost immediately and well before blood pressure had changed. This observation is consistent with results from animal studies\textsuperscript{19} and suggests an important role for endogenous NO generated from either eNOS or neuronal NOS in the control of heart rate in humans. The findings would not be consistent with the idea that cardiac changes were secondary to changes in blood pressure.

Increased plasma ADMA concentrations occur in a wide range of disease states or risk factors in which cardiovascular events are increased,\textsuperscript{2,8,11} and in some situations there is a clear relationship between the level of ADMA and morbidity or mortality.\textsuperscript{7} There is a strong biological rationale for assuming that these relationships are likely to be causal, because inhibition of NO enhances atherogenesis and cardiovascular disease in animal models.\textsuperscript{20,21} However, plasma ADMA levels seldom rise above the low micromolar range, and it is not clear that this would be sufficient to affect vascular function, particularly because concentrations of the NOS substrate arginine are much higher. Our results show that at a time when circulating concentrations of ADMA are in the order of 2 \(\mu\text{mol/L}\), substantial cardiovascular effects are evident and persistent. It is likely that plasma ADMA concentrations may have been higher immediately after the injection and before equilibration within the body compartments. However, because ADMA is generated within endothelial cells, intracellular concentrations of this compound are also higher than the overspill concentrations found in plasma.\textsuperscript{22,23} What the present study demonstrates is that the ADMA concentrations that have been measured in cardiovascular diseases can be associated with prolonged and major cardiovascular effects even in the presence of normal circulating levels of arginine. In future studies, it would be interesting to measure ADMA levels at different time points to assess peak levels and the half-life of this substance.

Plasma concentrations of ADMA are increased in patients with renal failure,\textsuperscript{2} and it is assumed that failed urinary clearance is a major mechanism underlying accumulation. However, ADMA also accumulates in many other diseases in which renal function is normal, and it is ADMA that rises rather than the biologically inactive SDMA.\textsuperscript{3} These observations have led to interest in the role of the enzyme DDAH as a mechanism to control ADMA levels. DDAH (of which there are 2 isoforms) metabolizes ADMA (but not SDMA) to dimethylamine and citrulline.\textsuperscript{24–27} In rats, more than 90% of ADMA is eliminated by the action of DDAH.\textsuperscript{24} Important differences in DDAH activity have been described across animal species,\textsuperscript{25–26} but there have been no studies of in vivo DDAH activity in humans to date. The present study shows a substantial increase in urinary dimethylamine excretion after administration of ADMA and is the first demonstration of DDAH activity in humans in vivo. The bulk of urinary dimethylamine (260 \(\mu\text{mol/d}\), as shown in this study and previous studies\textsuperscript{13,28}) is thought to arise from an endogenous source,\textsuperscript{29} suggesting that the rate of ADMA metabolism is in the order of 130 to 260 \(\mu\text{mol/d}\) compared with a renal excretion rate of approximately 60 \(\mu\text{mol/d}\).\textsuperscript{2} Consistent with these estimates, the average protein turnover is approximately 300 \(\text{g/d}\) and the average ADMA content is approximately 1 to 2 \(\mu\text{mol/g protein}\),\textsuperscript{30} giving a total body production of 300 \(\mu\text{mol ADMA}\) in 24 hours. Thus, a complete failure of DDAH activity (combined with a loss of renal excretion) would lead to a daily increase in plasma ADMA concentrations by approximately 5 \(\mu\text{mol/L}\). Oxidative stress, tumor necrosis factor \(\alpha\), homocysteine, and nitrosative stress have all been shown to reduce DDAH activity.\textsuperscript{4,31,32} We have shown previously that local inhibition of DDAH in the vessel wall increases ADMA accumulation and inhibits endothelium-dependent relaxation.\textsuperscript{33} The present finding of substantial ADMA metabolism to dimethylamine in humans provides evidence that DDAH is active in vivo and supports the suggestion that reduced DDAH activity attributable to oxidative stress or inflammation\textsuperscript{4,31,32} could raise circulating ADMA levels during disease.

ADMA is formed continuously and actively metabolized by 2 isoforms of DDAH that are widely expressed in vascular and nonvascular cells.\textsuperscript{27} This study demonstrates significant cardiovascular effects of exogenous ADMA and shows that these persist at a time when plasma concentrations may be considered rather low. Although we have only studied the acute effects of ADMA, it seems reasonable to assume that chronic exposure to raised ADMA is likely to produce even greater effects and may enhance atherogenesis.\textsuperscript{6,7,20,21} ADMA has recently been shown to be an independent and strong predictor of LV ejection fraction in patients with end-stage renal failure.\textsuperscript{11} The effects of ADMA on resting and exercise-stimulated cardiac output described here suggest a possible causal role for ADMA in the pathophysiology of heart failure as well as a mechanism for reduced exercise tolerance in other conditions. Finally, the profound effects on heart rate suggest that it would be interesting to determine whether ADMA levels correlate with heart rate changes or altered heart rate regulation in those conditions in which ADMA levels are increased.
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