Excessive Nitric Oxide Function and Blood Pressure Regulation in Patients With Autonomic Failure

Alfredo Gamboa, Cyndya Shibao, André Diedrich, Sachin Y. Paranjape, Ginnie Farley, Brian Christman, Satish R. Raj, David Robertson, Italo Biaggioni

Abstract—Approximately 50% of patients with autonomic failure (AF) suffer from supine hypertension, even those with very low plasma norepinephrine and renin. Because NO is arguably the most potent metabolic modulator of blood pressure, we hypothesized that impaired NO function contributes to supine hypertension in AF. However, we found that AF patients (n=14) were more sensitive to the pressor effects of the NO synthase inhibitor N\(^6\)-monomethyl-L-arginine, suggesting increased NO function rather than deficiency; a lower dose of N\(^6\)-monomethyl-L-arginine was needed to produce a similar increase in blood pressure in AF patients, as in healthy control subjects in whom AF was induced with the ganglionic blocker trimethaphan (171±37 mg versus 512±81 mg, respectively; P=0.001). Furthermore, potentiation of the actions of endogenous NO with the phosphodiesterase inhibitor sildenafil (25 mg PO) decreased nighttime supine systolic blood pressure from 182±11 to 138±4 mm Hg in 8 AF patients with supine hypertension (P=0.012 compared with placebo). Finally, AF patients tolerated a greater degree of upright tilt during infusion of N\(^6\)-monomethyl-L-arginine (56±6° versus 41±4° with placebo; n=7; P=0.014), an improvement in orthostatic tolerability similar to that obtained with equipressor doses of phenylephrine. In conclusion, AF patients do not have NO deficiency contributing to supine hypertension. Instead, they have increased NO function contributing to their orthostatic hypertensive state. Potentiation of NO could be used in the treatment of supine hypertension, and its inhibition offers a novel approach to improve orthostatic hypotension. (Hypertension. 2008;51:1531-1536.)

Key Words: NO ■ orthostatic hypotension ■ supine hypertension ■ pure autonomic failure ■ Shy-Drager syndrome ■ blood pressure ■ L-NMMA

Autonomic failure is characterized by severe orthostatic hypotension that can occur from an autonomic neuropathy secondary to systemic illnesses, such as diabetes mellitus or amyloidosis, or as a primary neurodegenerative disorder. The primary forms include pure autonomic failure (PAF), which presents only with autonomic nervous system manifestations, and multiple system atrophy (MSA; Shy-Drager syndrome), which is associated with a movement disorder or truncal ataxia in addition to autonomic function (AF). Both MSA and PAF patients are characterized clinically by disabling orthostatic hypotension, as would be expected from their severe AF. In addition, ≈50% of patients with autonomic impairment because of either PAF or MSA suffer from supine hypertension, which can be severe, with systolic blood pressure (SBP) in many cases >200 mm Hg.\(^1\) In the case of MSA, we have shown previously that hypertension may be explained by residual sympathetic tone, possibly acting on hypersensitive adrenoreceptors and unrestrained by the lack of baroreflex modulation.\(^2\) In contrast, the cause of hypertension in PAF remains unknown. It is important to note that hypertension in these patients is because of an increase in vascular resistance,\(^3\) despite having very low plasma norepinephrine and renin activity.\(^4\) Therefore, the driving force for this increased vascular tone is not known but is likely to be magnified by the lack of baroreflex buffering capacity resulting from their AF.

Because autonomic neural mechanisms do not explain the hypertension of PAF, it is likely that hormonal or metabolic factors are involved. We have shown recently that NO is arguably the most important metabolic regulator in normal subjects, tonically restraining blood pressure \(≈30\) mm Hg.\(^5\) NO deficiency has been proposed to play a role in essential hypertension\(^6\) and other cardiovascular disorders.\(^7\) We, therefore, hypothesized that the hypertension of PAF is because of impaired NO. Our results, however, suggest that AF is characterized by excess NO function rather than a deficiency and that this excess may contribute to the orthostatic hypotension in these patients.

Materials and Methods

Subjects

We studied 20 patients with AF. Fourteen were diagnosed with PAF (age: 67.0±2.5 years; 10 men), and 6 were diagnosed with...
MSA (age: 60.0 ± 3.6 years; 4 men). Patients were diagnosed following the criteria of the American Autonomic Society to differentiate between MSA and PAF. Patients were excluded if they had secondary forms of AF (eg, diabetes mellitus or amyloidosis) or renal failure. Subjects could be included in >1 protocol. Fourteen subjects participated in study 1 (to determine blood pressure response to systemic NO synthase inhibition), 8 patients participated in study 2 (to evaluate the effects of NO potentiation on supine hypertension of AF), and 7 subjects participated in study 3 (to determine whether systemic NO synthase inhibition improves orthostatic intolerance comparable to α-adrenergic activation). All of the studies were approved by the institutional review board at Vanderbilt University.

General Protocol
Patients were admitted to the Vanderbilt University Medical Center General Clinical Research Center. Medications with cardiovascular/autonomic effects were discontinued for ≥5 half-lives before admission. Patients were placed in a metabolic ward on a sodium-balanced diet. The diet consisted of low monoamine, caffeine-free food, containing 150 milliequivalents of sodium and 70 milliequivalents of potassium per day. Studies were conducted ≥2.5 hours after a meal. The screening consisted of a comprehensive medical history, physical examination, 12-lead ECG, and laboratory assessments. Standardized autonomic function tests were performed to access the severity of autonomic impairment. These included an orthostatic stress test, Valsalva maneuver, the cold pressor test, handgrip, and sinus arrhythmia, as described previously. Brachial blood pressure and heart rate during all of these tests were obtained using an automated cuff-oscillometric sphygmomanometer (Dinamap). Laboratory Measurements Plasma norepinephrine levels were determined by high-performance liquid chromatography with electrochemical detection. In a subset of AF patients (n = 12) and normal control subjects (n = 11), baseline determination of NO products (NOx) was performed using the chemiluminescence assay. This was performed in plasma samples taken at the initial evaluation and kept frozen at −80°C until the time of analysis. The chemiluminescence assay was done after reduction to NO by using a vanadium HCl catalyst. The NO generated was reacted in an ozone chamber and detected by chemiluminescence in an NO analyzer (Sievers, Medical Systems Information Technologies).

Study 1: To Determine Blood Pressure Response to Systemic NO Synthase Inhibition in Patients With AF
The studies were conducted in the morning with the patient in the recumbent position ≥8 hours after their last meal. Heart rate was determined with continuous ECG monitoring and blood pressure through the volume clamp method (Finapres 2300, Ohmeda) and also an automated brachial cuff-oscillometric sphygmomanometer (Dinamap). An intravenous line for N\textsuperscript{\textdagger}m-monomethyl-L-arginine (l-NMMA) infusion was placed in a large antecubital vein in the left arm.

After a stable baseline was reached, patients were gradually tilted head up until an SBP of ~110 mm Hg was reached. After stabilization at this new baseline, l-NMMA was infused at 4 different doses for 15 minutes each (33, 83, 167, and 250 µg/kg per minute) or until SBP reached 150 mm Hg.

For comparison, we used a group of 10 healthy control subjects who were subjected to transient pharmacological autonomic withdrawal with trimethaphan, as described previously. Briefly, an intravenous line with 3 infusion ports connected to the catheter was placed in a large antecubital vein in the left arm; 1 port was for trimethaphan infusion, the second for infusion of phenylephrine, and the third for l-NMMA. After a stable baseline was reached, N\textsuperscript{\textdagger}m-cholinergic receptors were blocked by continuous infusion of trimethaphan (Cambridge Pharmaceuticals) at 4 µg/min. We have shown previously that this dose induces virtually complete autonomic blockade and a mild but significant lowering of blood pressure. Blood pressure was then restored to pretrimethaphan levels (or increased up to an SBP of 110 mm Hg if baseline levels were >120 mm Hg) by infusing phenylephrine at individually titrated doses, starting with 0.05 µg/kg per minute. l-NMMA was then infused at 2 different doses for 15 minutes each (250 and 500 µg/kg per minute) or until SBP reached 150 mm Hg.

Study 2: To Evaluate the Effects of NO Potentiation on Supine Hypertension of AF
We compared the effect of 25 mg of sildenafil with placebo on overnight supine blood pressure in 8 patients with AF and supine hypertension. The order of the intervention was randomized. The medication was administered orally with 50 mL of tap water at 8:00 PM and ≥2.5 hours after the last meal. Patients were instructed to remain supine throughout the night, fluid intake was restrained, and blood pressure was measured at 2-hour intervals by an automated sphygmomanometer.

All of the patients were men with primary AF. All of the patients had nighttime supine hypertension defined as supine SBP >150 mm Hg and/or supine diastolic blood pressure >90 mm Hg.

Study 3: To Determine Whether Systemic NO Synthase Inhibition Improves Orthostatic Intolerance Comparable to α-Adrenergic Activation
Seven AF patients were studied on 2 separate study days randomly assigned ≥1 day apart using a crossover design. On both occasions, orthostatic tolerance to passive head-up tilt was tested before and after either NOS blockade or α-adrenergic activation.

The studies were conducted in the morning with the subject in the recumbent position ≥8 hours after their last meal. Heart rate was determined with continuous ECG monitoring, blood pressure through the volume clamp method (Finapres 2300, Ohmeda), and also automated brachial cuff-oscillometric sphygmomanometer (Dinamap). One intravenous line was connected to a catheter placed in a large antecubital vein in the left arm for infusion of l-NMMA (NO day) or phenylephrine (phenylephrine day).

In both days, an orthostatic tolerance test was performed at baseline before any drug was administered. The orthostatic tolerance test consisted of a graded head-up tilt at 5° intervals for 4 minutes at each degree, until patients reached an SBP of <70 mm Hg or developed symptoms of presyncope. Subjects were then returned to the supine position.

After a resting period, subjects were then gradually tilted head up until an SBP of ~110 mm Hg was induced and, after stabilization, new baseline measurements were taken. l-NMMA was infused at increasing doses for 15 minutes each (33, 83, 167, and 250 µg/kg per minute) or until SBP reached 150 mm Hg. The orthostatic tolerance test performed at the beginning of the study was then repeated. A similar protocol was followed on the phenylephrine day, but instead of l-NMMA, subjects received phenylephrine starting at 0.1 µg/kg per minute and increasing it every 6 minutes until SBP reached 150 mm Hg.

Statistical Analysis
For study 1, the primary outcome of interest was the cumulative dose of l-NMMA needed to increase SBP to 150 mm Hg. The study design used 2 parallel groups (AF patients and healthy control subjects). Differences between group means were tested by the Mann–Whitney U test. In study 2, the outcome of interest was nighttime SBP measured every 2 hours from 8 PM to 8 AM in a crossover design (the same subject was studied twice, once while receiving placebo and on a different occasion after receiving silde-

The area under the curve for SBP overnight was used to summarize the data, and the difference between treatments was analyzed with the Wilcoxon signed-rank test. In study 3, the primary outcome of interest was the degree of tolerance to head-up tilt during NOS inhibition with l-NMMA or during α-stimulation with phen-
ylephrine at similar SBP levels. Kaplan-Meier curves were compared using the log-rank test.

Data are reported as means±SEMs. All of the tests were 2-tailed. A value of $P<0.05$ was considered significant. Statistical analyses were performed using SPSS for Windows version 15.0 (SPSS Inc).

Results

General

Hemodynamic responses to posture are shown in the Table. Mean SBP and diastolic blood pressure were $162±11/90±6$ mm Hg while supine and decreased to $79±6/52±4$ mm Hg on standing, respectively. The compensatory increase in heart rate was inadequate considering the profound decrease in blood pressure, indicating failure in baroreflex modulation. Supine plasma norepinephrine was lower in PAF patients compared with MSA (93±17 and 174±23 pg/L, respectively) but did not increase adequately on standing in either group (168±32 and 410±152 pg/mL), considering the severity of orthostatic hypotension. SBP decreased significantly during phase II of the Valsalva maneuver (from $160±11$ to $104±14$ mm Hg), and the expected phase IV blood pressure overshoot was absent. Both findings indicate impaired sympathetic function. Respiratory sinus arrhythmia was reduced ($1.1±0.01$ mm Hg; normal values: $>1.2$ mm Hg) consistent with parasympathetic dysfunction. Furthermore, the blood pressure response to the cold pressor test was blunted ($9±3$ mm Hg; normal: $>20$ mm Hg). Plasma NOx concentrations were similar between AF patients and control subjects ($30.9±5.2$ μmol/L for AF patients and $32.4±6.9$ μmol/L for control subjects; $P=0.928$ by Mann–Whitney U test).

Study 1: AF Patients Are More Sensitive to NOS Inhibition, Suggesting Increased Endogenous NO Function

AF patients were subjected to gradual head-up tilt until an SBP of $110$ mm Hg was reached, whereas healthy control subjects received trimethaphan to induce a transient AF. This paradigm resulted in similar “baseline” blood pressures (Figure 1A). L-NMMA was then infused at increasing doses individualized to increase SBP to $150$ mm Hg. As expected, the changes in SBP were very similar in both groups (from $108±2$ to $14±4$ mm Hg in AF patients and control subjects, respectively). The cumulative dose of L-NMMA required to increase SBP was $171±10$ mg in patients with AF, whereas in healthy control subjects the dose needed was $512±24$ mg (Figure 1B; $P=0.001$ by Mann–Whitney U test). This result suggests that, instead of NO impairment, AF patients have a greater NO function. We also subdivided our AF patients between those with or without supine hypertension to further determine whether relative NO deficiency contributed to supine hypertension (Figure 2). In 9 patients with supine hypertension (SBP=$174±4$ mm Hg) and 5 without supine hypertension (SBP=$124±6$ mm Hg), the cumulative total amount of L-NMMA needed to increase SBP to $150$ mm Hg was similar between groups ($P=0.438$ by Mann–Whitney U test). SBP increased from $107±2$ to $154±5$ mm Hg and from $111±2$ to $148±5$ mm Hg in patients with and without supine hypertension, respectively.

Table. Baseline Characteristics

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Study 2: NO Potentiation With Sildenafil Controls Supine Hypertension in AF
During the placebo night, SBP decreased from 181±9 mm Hg at 8 PM to 177±8 mm Hg at 2 AM. Sildenafil produced a significantly larger decrease in SBP, from 182±11 to 138±4 mm Hg (Figure 3; P=0.012 for the differences in AUC between treatments by Wilcoxon signed-rank test). SBPs were similar between groups after these interventions. The NO synthase inhibitor L-NMMA was then infused at increasing doses, individualized to reach an SBP of 150 mm Hg. B, Cumulative total amount of L-NMMA required to increased SBP to 150 mm Hg in AF patients (□) and normotensive controls (NLS; □).

Study 3: Systemic NOS Inhibition Improves Orthostatic Tolerance in AF
AF patients were subjected to graded head-up tilt to presyncope or SBP <70 mm Hg, before and after systemic administration of L-NMMA or phenylephrine at doses titrated to achieve similar SBPs. On the L-NMMA day, tilt had to be stopped at 41±4° at baseline, but during L-NMMA infusion, patients were able to tolerate a higher head-up tilt (56±6°; Figure 4A; P=0.014). On the phenylephrine day, orthostatic tolerance increased from 40±4° to 59±5° (Figure 4B; P=0.005). No differences were found for the survival to tilt between phenylephrine and L-NMMA (P=0.9786 by the log-rank test).

Discussion
Our results indicate that, contrary to our original hypothesis, AF patients have increased NO function. This conclusion is based on 2 complementary observations: AF patients had a...
greater pressor response to NO synthase inhibition and also had an exaggerated depressor response to NO function augmentation with sildenafil.

We found that lower doses of the NOS inhibitor L-NMMA were required to produce similar increases in blood pressure in AF compared with normal subjects in whom AF was induced with the ganglionic blocker trimethaphan. If AF patients had an impaired NO function, we would have seen the opposite response, a blunted pressor response to L-NMMA. This is precisely what we observed, using a very similar approach, in smokers, a patient population widely accepted to have an impaired NO function. The greater pressor response to NO function contributing to their orthostatic hypotension. Potentially, AF patients also have parasympathetic impairment, and cholinergic blockade has been shown to markedly potentiate the pressor effects of L-NMMA.

We also found that L-NMMA produced similar pressor responses in AF patients with or without supine hypertension. We conclude, therefore, that endogenous NO deficiency does not contribute to the supine hypertension of AF. On the contrary, AF patients have supine hypertension despite having excess NO function. Thus, increased NO function is not sufficient to prevent hypertension. Conversely, we and others have observed previously that NO deficiency, as seen in heavy smokers, is not sufficient to induce hypertension.

Thus, despite the potent effect that endogenous NO has in the acute regulation of blood pressure, its role in sustaining hypertension in humans is not certain. We should note that we have only examined the NO function in patients with impaired autonomic function and have not evaluated NO/autonomic interactions, which could also contribute to hypertension.

The driving force for supine hypertension in PAF remains unknown. On the other hand, we can use their NO function excess to our advantage in the treatment of supine hypertension. We found that potentiating NO signaling with the phosphodiesterase inhibitor sildenafil resulted in a significant lowering of nighttime blood pressure. Because sildenafil acts by enhancing endogenous NO function, the opposite would have been observed if patients had impaired NO function; eg, sildenafil is less effective in improving endothelium-dependent vascular responses in smokers. Thus, the exaggerated hypotensive effect observed in our patients also supports the concept that AF is associated with increased NO function.

In contrast to the lack of effect of tonic NO in supine hypertension, inhibition of NO production with L-NMMA significantly improved orthostatic tolerance in AF patients. At baseline, none of our patients was able to tolerate a 70° head-up tilt, but 50% of patients were able to tolerate this after NOS blockade. This improvement in orthostatic tolerance was similar to that obtained by restoring noradrenergic receptor stimulation with phenylephrine.

Our results cannot differentiate between increased NO production or increased sensitivity to NO actions. The fact that similar baseline plasma NOx values were seen in AF patients and control subjects raises the possibility that the observed increase in NO function may be explained by an increased sensitivity to NO signaling. In this regard, AF patients also have an exaggerated depressor response to NO donors, such as nitroglycerine. A caveat to this conclusion is the known limitations in estimating NO production in vivo.

In conclusion, contrary to our original hypothesis, AF patients do not have NO deficiency contributing to supine hypertension. On the contrary, they have increased NO function contributing to their orthostatic hypotension. Potentiation of NO could be used in the treatment of supine
hypertension, and its inhibition offers a novel approach to improve the orthostatic intolerance of AF.

**Perspectives**

Patients with AF provide a unique model of a condition characterized by excess NO function. AF patients with and without supine hypertension appear to have similar excess NO function, questioning the role of this autacoid in long-term blood pressure regulation. On the other hand, our finding that autonomic impairment is associated with greater NO function is in agreement with previous observations, suggesting that transient increases in sympathetic tone impair NO function. Taken together, these observations suggest reciprocal modulation of NO by the autonomic nervous system.

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**Disclosures**

None.

**References**

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