Nitric Oxide and Regulation of Heart Rate in Patients With Postural Tachycardia Syndrome and Healthy Subjects

Alfredo Gamboa, Luis E. Okamoto, Satish R. Raj, André Diedrich, Cyndya A. Shibao, David Robertson, Italo Biaggioni

Abstract—The objective is to study the role of nitric oxide (NO) on cardiovascular regulation in healthy subjects and postural tachycardia syndrome (POTS) patients. Reduced neuronal NO function, which could contribute to a hyperadrenergic state, and increased NO-induced vasodilation, which could contribute to orthostatic intolerance, have been reported in POTS. In protocol 1, 13 healthy volunteers (33±3 years) underwent autonomic blockade with trimethaphan and were administered equipressor doses of Nω-monomethyl-L-arginine (L-NMMA, a NO synthase inhibitor) and phenylephrine to determine the direct chronotropic effects of NO (independent of baroreflex modulation). In protocol 2, we compared the effects of L-NMMA in 9 POTS patients (31±3 years) and 14 healthy (32±2 years) volunteers, during autonomic blockade. During autonomic blockade, L-NMMA and phenylephrine produced similar increases in systolic blood pressure (27±2 versus 27±3 mm Hg). Phenylephrine produced only minimal heart rate changes, whereas L-NMMA produced a modest, but significant, bradycardia (–0.8±0.4 versus –4.8±1.2 bpm; P=0.011). There were no differences between POTS and healthy volunteers in the systolic blood pressure increase (22±2 and 28±5 mm Hg) or heart rate decrease (–6±2 and –4±1 bpm for POTS and controls, respectively) produced by L-NMMA. In the absence of baroreflex buffering, inhibition of endogenous NO synthesis results in a significant bradycardia, reflecting direct tonic modulation of heart rate by NO in healthy individuals. We found no evidence of a primary alteration in NO function in POTS. If NO dysfunction plays a role in POTS, it is through its interaction with the autonomic nervous system. (Hypertension. 2013;61:00-00.)

Key Words: autonomic blockade ■ autonomic nervous system ■ blood pressure ■ heart rate ■ nitric oxide ■ postural tachycardia syndrome

Postural tachycardia syndrome (POTS) is characterized by an excessive increase in heart rate (HR; >30 bpm) on assuming the upright posture, associated with symptoms suggestive of cerebral hypoperfusion or sympathetic activation, but in the absence of orthostatic hypotension.1 POTS is an important cause of disability in otherwise healthy young adults, disproportionately affecting women of childbearing age.2 The pathophysiology of POTS is not completely understood. Increased sympathetic activity appears to play a significant role, but the primary cause of this hyperadrenergic state is not clear.

Nitric oxide (NO) is one of the most important metabolic modulators of blood pressure and cardiovascular function. Our previous studies suggest that NO tonically restrains blood pressure by at least 30 mm Hg in healthy subjects.3 The mechanisms by which NO tonically decreases blood pressure include a direct vasodilatory action4 and inhibition of sympathetic nervous system tone.5,6 Given its importance in cardiovascular regulation, the recent interest in the role of NO in POTS is not surprising. Both excessive NO-mediated dilation7 and decreased neuronal NO activity8 have been reported in POTS. Given the evidence suggesting that NO tonically inhibits sympathetic tone, a decrease in NO function in POTS could contribute to the hyperadrenergic state seen in this disorder. Conversely, an increase in NO-induced vasodilation could contribute to orthostatic intolerance, as seen in patients with autonomic failure.9 It is also possible that NO deficiency contributes to tachycardia. Both a direct effect of NO on the sinoatrial node function and an indirect effect through modulation of autonomic control of HR have been reported.10 The mechanism of this putative effect of NO on HR modulation is not yet fully understood,11 but all 3 known genomic isoforms of NO synthase (NOS) are reportedly present and functionally active in the heart,12 and NO synthase is expressed in the sinoatrial node.13

We have developed an in vivo research model that allows us to determine whether there is an intrinsic NO deficiency in humans, independent of its interaction with the autonomic nervous system.3 We used autonomic ganglionic blockade, with the Nω-nicotinic receptor antagonist trimethaphan, to eliminate the restraining effect of the baroreflex, and thus allow for the full expression of the effect of NOS inhibition. This model also removes any potential interactions between NO and the autonomic nervous system. Under these experimental conditions, L-NMMA produced a significant bradycardia, reflecting direct tonic modulation of heart rate by NO in healthy individuals. We found no evidence of a primary alteration in NO function in POTS. If NO dysfunction plays a role in POTS, it is through its interaction with the autonomic nervous system.

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conditions, the decrease in HR induced by systemic NOS inhibition can be attributed to direct chronotropic effects of NO. Similarly, any increase in blood pressure can be attributed to inhibition of the tonic vasodilatory effects of NO.

In protocol #1, we tested the direct chronotropic effect of NO, independent of neural modulation or systemic hemodynamic changes. We compared the effect on HR of equipressor doses of L-NMMA (NOS inhibitor) and phenylephrine (α1-adrenergic agonist) in healthy subjects during autonomic blockade. In protocol #2, we compared the effect of L-NMMA on HR and blood pressure during autonomic blockade in patients with POTS, and age- and sex-matched healthy subjects. If POTS patients have excessive NO function, this would be manifested as augmented bradycardia and pressor response to L-NMMA.

**Methods**

**Subjects**

POTS patients were enrolled from those referred to the Vanderbilt University Autonomic Dysfunction Center. Patients met criteria for POTS if they developed symptoms of orthostatic intolerance accompanied by a HR rise ≥20 bpm within the first 10 minutes of standing, in the absence of orthostatic hypotension (a fall in blood pressure ≥20/10 mmHg).14 All patients had symptoms for at least 6 months in the absence of an additional chronic debilitating disorder or prolonged bed rest, were aged at least 18 years, were free of medications that could impair autonomic function, and were not taking fludrocortisone for at least 5 days before testing.

Healthy volunteers were recruited from the Vanderbilt University Clinical Research Center volunteer database and Research Match,15 a national register to recruit volunteers for clinical trials. Subjects aged 18 to 60, both men and women, with a body mass index between 20 and 40 kg/m² were considered eligible for the study. Subjects were non-smokers, and were otherwise healthy (no concomitant cardiovascular or other diseases). All subjects were asked to avoid pregnancy for the duration of the study. Pregnancy was excluded in women subjects with a pregnancy test. Women taking oral contraceptives were allowed to continue using them.

Subjects abstained from caffeine for ≥72 hours before testing. During the screening visit, all subjects underwent a clinical examination, ECG, and admission urinalysis and routine laboratory test.

The study was reviewed and approved by the Vanderbilt University Institutional Review Board, and written informed consent was obtained from each subject before initiating the study. Before enrolling, the study was registered in clinicaltrials.gov.

**General Study Design**

The studies were conducted in the morning with the subject fasting (28 hours after their last meal), while lying supine. Blood pressure was measured by the volume clamp method (Finapres 2300: Ohmeda), and also with an automated oscillometric brachial cuff (Vital-Guard 450C, Ivy Biomedical Systems). HR was determined from continuous ECG monitoring. An intravenous line was inserted in the nondominant arm in a large antecubital vein. Three infusion ports were connected to this catheter, one for trimethaphan infusion, the second for infusion of phenylephrine, and the third for L-NMMA. A saline lock was placed in the other arm to administer phenylephrine.

After a 15-minute baseline recording, trimethaphan 4 mg/min (Cambridge Pharmaceuticals) was infused, and this was continued throughout the study to assure complete autonomic blockade. 3,16 Effectiveness of autonomic blockade was verified by phenylephrine bolus injections. Briefly, after a new stable blood pressure was achieved, phenylephrine bolus injections commenced starting at a dose of 2.5 µg. The phenylephrine bolus was repeated every 3 to 5 minutes with an increasing dose until systolic blood pressure (SBP) increased ≥20 mm Hg. The absence of reflex bradycardia in response to the increase in blood pressure was taken as evidence of effective autonomic blockade.

Intrinsic blood pressure (in the absence of autonomic influences) was recorded, and then SBP was clamped at ≥110 mm Hg by titrating phenylephrine infusions starting at a rate of 0.05 µg/kg per min. This allowed us to study all subjects at similar clamped adrenergic tone and a comparable starting blood pressure. Once blood pressure was stable and no more adjustments in phenylephrine dose were needed, a new baseline period was recorded for 15 minutes. Systemic inhibition of NOS with L-NMMA (Bachem, Switzerland) was then started and continued throughout the study. The doses of L-NMMA range from 125 to 250 µg/kg per min. Each dose was maintained for 15 minutes. Blood pressure was measured every 2 minutes for the first 8 minutes and every 1 minute for the last 7 minutes, when a stable recording was achieved. The last 5 measurements after stable recording were used for the analysis.

**Protocol 1: NO Effects on HR Regulation During Autonomic Blockade in Healthy Controls**

Thirteen healthy controls were studied in this protocol (33±3 years; body mass index=25±1 kg/m², 8 women). All procedures were identical, as described for the general protocol.

After autonomic blockade with trimethaphan was achieved, we targeted for an increased systolic blood pressure of ≥25 mm Hg, either with L-NMMA 250 µg/kg per min for systemic NOS inhibition or with boluses of phenylephrine (25–50 µg) for direct α-adrenergic activation.

**Protocol 2: NO Effects on HR and Blood Pressure Regulation in POTS Patients**

A parallel group design was used to compare the effects of NOS inhibition between healthy subjects and POTS patients. Thirteen healthy controls (all women) and 9 POTS patients (all women) were included in this study. Instrumentation and pharmacological testing were identical to those described for the general protocol.

The dose of L-NMMA ranged from 125 to 250 µg/kg per min depending on the individual increase on systolic blood pressure. For safety reasons, we did not increase the dose of L-NMMA if a SBP of >150 mm Hg was already achieved. The main comparison was the change in systolic blood pressure at the dose of 250 µg/kg per min.

**Data Acquisition**

The surface ECG lead II was amplified, and no additional filters were applied. ECG and blood pressure were digitized with 14-bit resolution and 500-Hz sample frequency, and recorded using the WINDAQ data-acquisition system (DATAQ). Data were analyzed offline using a customized program for data analysis written in PV-Wave (VNI), by one of the authors (A.D.).

**HR Variability and Blood Pressure Variability**

A QRS detection algorithm, modified from Pan and Tompkins,17 was used to generate beat-to-beat values. The nonequidistant event time series of RR intervals and blood pressure values were interpolated, low-pass filtered (cutoff: 2 Hz), and resampled at 4 Hz. Data segments of 300 seconds, recorded during stable resting conditions, were used for spectral analysis. Linear trends were removed, and power spectral density was estimated with the fast Fourier transform-based Welch algorithm, using segments of 256 data points with 50% overlapping and Hanning window.18 The Hanning window was applied to previous estimation of the power spectral density. The power in the frequency ranges for very low frequencies (0.003 to <0.04 Hz), low frequencies (0.04 to <0.15 Hz), and high frequencies (0.15 to <0.40 Hz) were calculated for each interval, according to Task Force recommendations.19
Statistical Analysis
Data are presented as mean±SEM. Comparison of outcomes by interventions in the same subjects were performed by Wilcoxon signed-rank test, and comparisons of demographics and outcomes by groups were performed using Mann–Whitney U test.

For protocol 1, the null hypothesis was that, given the same increase in systolic blood pressure, there would be no difference in HR responses to either phenylephrine or L-NMMA during autonomic blockade. The primary end point for protocol 1 was the decrease in HR produced during autonomic blockade and NOS inhibition.

For protocol 2, the null hypothesis was that there would be no difference in outcomes in the response to systemic infusion of L-NMMA between POTS patients and healthy subjects. The primary outcome for protocol #2 was the change in HR produced by NOS inhibition during autonomic blockade in POTS patients and healthy subjects. The change in SBP during the same period was a secondary end point.

All of the tests were 2-tailed, and a probability value of <0.05 was considered significant. Analyses were performed with the SPSS statistical software (SPSS version 19.0, SPSS Inc).

Results
We studied a total of 28 subjects. Thirteen healthy volunteers (5 men) were included in protocol 1. Nine POTS patients and 14 healthy, sex- and age-matched volunteers were included in protocol 2. All 8 healthy women that participated in protocol 1 also participated in protocol 2. Demographics, baseline characteristics, and response to orthostatic stress test are described in Table 1. We included normative data for catecholamines from our laboratory to serve as a reference to compare orthostatic changes in POTS patients.

The orthostatic stress test produced significant changes in HR and diastolic blood pressure in all 3 groups (P<0.05 when compared with supine; Table 1). The increase in HR was more dramatic among POTS patients (15±2, 17±2, and 52±4 bpm, for healthy controls in protocol 1, controls in protocol 2, and POTS patients, respectively; Table 1, P<0.001

Table 1. Baseline Characteristics of All Participants

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Protocol 1</th>
<th>Protocol 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Controls</td>
<td>POTS</td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=14)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>Age, y</td>
<td>33±3</td>
<td>32±2.4</td>
<td>31±3.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±1.2</td>
<td>24±1.0</td>
<td>23±1.1</td>
</tr>
<tr>
<td>Sex (women/total)</td>
<td>8/13</td>
<td>14/14</td>
<td>9/9</td>
</tr>
<tr>
<td>LF RRI, ms²</td>
<td>1097±298</td>
<td>1527±446</td>
<td>534±170</td>
</tr>
<tr>
<td>HF RRI, ms²</td>
<td>1256±446</td>
<td>1469±423</td>
<td>427±173</td>
</tr>
<tr>
<td>HF RRI/LF RRI, ms²</td>
<td>1.51±0.33</td>
<td>1.42±0.31</td>
<td>1.74±0.36</td>
</tr>
<tr>
<td>LF SBP, mmHg</td>
<td>6.89±1.85</td>
<td>4.92±0.93</td>
<td></td>
</tr>
<tr>
<td>Orthostatic stress test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>110±3.51</td>
<td>107±2.96</td>
<td>107±5.3</td>
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<tr>
<td>DBP, mmHg</td>
<td>64±2.17</td>
<td>65±2.22</td>
<td>69±2.79</td>
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<tr>
<td>HR, bpm</td>
<td>65±2.3</td>
<td>68±2.47</td>
<td>71±1.93</td>
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<tr>
<td>Standing</td>
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<tr>
<td>SBP, mmHg</td>
<td>113±3.44</td>
<td>109±3.29</td>
<td>115±6.58</td>
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<tr>
<td>DBP, mmHg</td>
<td>73±2.55*</td>
<td>74±2.50*</td>
<td>74±2.77*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>80±3.27*</td>
<td>86±3.13*</td>
<td>123±5.08*</td>
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<tr>
<td>∆SBP</td>
<td>2±2.13</td>
<td>2±2.4</td>
<td>8±3.9</td>
</tr>
<tr>
<td>∆DBP</td>
<td>9±1.22</td>
<td>9±1.4</td>
<td>6±2.1</td>
</tr>
<tr>
<td>∆HR</td>
<td>15±2.11</td>
<td>17±1.9</td>
<td>52±4.4</td>
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<tr>
<td>Plasma norepinephrine (pg/mL)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>186±13</td>
<td>256±83</td>
<td></td>
</tr>
<tr>
<td>Upright</td>
<td>397±18*</td>
<td>666±104*</td>
<td></td>
</tr>
<tr>
<td>∆NE (standing-supine)</td>
<td>211±19.8</td>
<td>411±109</td>
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<tr>
<td>Plasma epinephrine (pg/mL)†</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>15±2.4</td>
<td>27±6.3</td>
<td></td>
</tr>
<tr>
<td>Upright</td>
<td>39±9.0*</td>
<td>96±29.5*</td>
<td></td>
</tr>
<tr>
<td>∆Epi (standing-supine)</td>
<td>24±8.5</td>
<td>69±30.9</td>
<td></td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HF, high frequency; HR, heart rate; LF, low frequency; POTS, postural tachycardia syndrome; RRI, R-R heart rate interval; and SBP, systolic blood pressure.

Values are presented as mean±SEM.

P<0.05 when compared with supine.

†Controls values from the Autonomic Dysfunction Database at Vanderbilt University presented as a reference.
Our predetermined systolic blood pressure safety limit of 150 mm Hg. To account for this, we gave a dose of 125 µg/kg per min to 3 control subjects. Cumulative doses of L-NMMA were not different between POTS and controls (219±18 and 253±34 mg; P = 0.516). The maximal tolerated dose of L-NMMA produced an increase in SBP in POTS (ΔSBP_{L-NMMA} = 28±5 mm Hg) that was similar to equivalent doses in controls (22±2 mm Hg; P = 0.688). Increases in diastolic blood pressures were also similar (ΔDBP_{L-NMMA} = 22±4 and 18±4 mm Hg; P = 0.224). HR decreased to a similar extent in both POTS and controls (ΔHR_{L-NMMA} = -5.9±1.7 versus -4.4±1.3 bpm; P = 0.477).

### Discussion

There are 2 main new findings in the present study. The first one is that, after removing autonomic influences, inhibition of endogenous NO synthesis with L-NMMA results in significant bradycardia. This effect is not explained by its pressor effect or baroreflex responses, as the same increase in SBP with phenylephrine did not reduce HR during autonomic blockade. These data show that NO has a tonic effect on human HR in vivo, and that this effect is independent of the autonomic
control of HR. It is well known that NO is produced within the heart not only by endothelial cells, but also by myocytes.\textsuperscript{11,22} It has been proposed that NO has a role in the regulation of cardiac function both under normal and pathological conditions.\textsuperscript{11,22,23} NOS inhibition was previously shown to reduce HR in patients with transplanted, and presumably denervated, hearts.\textsuperscript{24} Our findings indicate for the first time that endogenous NO tonically regulates HR in healthy subjects. This is also in agreement with studies in animal models and isolated human hearts, suggesting that NO provides a direct positive chronotropic effect. The present study, therefore, contributes to our understanding of HR modulation by NO without the confounding effect of the baroreflex.

The second new finding is that NO function is not altered in POTS patients. After removing autonomic influences, the effects of NOS inhibition on HR and blood pressure were comparable between POTS patients, and sex- and age-matched controls. This finding indicates a lack of an intrinsic impairment of NO function in POTS patients. If endogenous NO were to contribute to the tachycardia observed in POTS, then NOS inhibition would result in a greater reduction of HR in this patient population, which was not observed in this study. One could also speculate that excessive NO-mediated vasodilation could contribute to tachycardia in POTS through reflex mechanisms. If indeed endogenous NO did cause greater vasodilation in POTS patients, then systemic NOS inhibition would result in a greater increase in SBP for this group. We did find a nonsignificant trend toward an augmented blood pressure response in POTS with NOS inhibition, but the magnitude of this effect is of questionable biological significance. Assuming that the point estimates from this study are correct, we would have needed at least 120 POTS patients, and an equal number of healthy subjects for this difference to reach statistical significance with 90% power.

It remains possible that a decrease in neuronal NO could contribute to the sympathetic activation seen in POTS. Conversely, sympathetic activation can induce a secondary impairment in NO function. The current experimental paradigm does not allow us to tease out these potential interactions between NO and the autonomic nervous system.

Finally, this study confirms previous findings\textsuperscript{22} that POTS patients have a greater reliance of sympathetic activity to maintain their blood pressure, as evidenced by their greater fall in systolic blood pressure during autonomic blockade. Intrinsic blood pressure (in the absence of autonomic influences) was about 9 mm Hg lower in POTS than healthy subjects (86±4 versus 99±3; \( p = 0.039 \)). The cause of this constitutional hypotension remains unknown. Our findings raise the possibility that sympathetic activation in POTS is a compensatory response to these putative mechanisms.

In conclusion, we found that when disengaged from the autonomic nervous system, NO has a positive tonic effect on HR in both POTS patients and in healthy subjects. There were no differences in the response to NOS inhibition in POTS patients compared with healthy controls, arguing against a primary NO impairment contributing to the pathophysiology of POTS. If NO dysfunction plays a role in POTS, it is likely through its interactions with the autonomic nervous system.

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Disclosures
None.

References


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**What Is New?**

- Nitric oxide synthase has been shown to be present in the heart and proposed to play a role in the regulation of cardiac function. This is the first study to show that after removal of autonomic influences, NOS inhibition results in a significant drop in heart rate, implying a direct positive chronotropic effect in humans.

- It has been postulated that patients with postural tachycardia syndrome (POTS) have an altered nitric oxide (NO) function. This is the first study of the effects of systemic NOS inhibitions in POTS patients.

**What Is Relevant?**

- These data reveal in vivo, the importance of NO on regulation of heart rate in humans.

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**Novelty and Significance**

- In this study, we did not find differences between POTS patients and controls in their response to NOS inhibition. If NO dysfunction plays a role in POTS, it is likely through its interactions with the autonomic nervous system.

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**Summary**

The present study found that when disengaged from the autonomic nervous system, NO has a positive tonic effect on heart rate in both healthy subjects and POTS patients. We found no evidence of a primary alteration of NO function in POTS.
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