L-Arginine Augments Cardiac Vagal Control in Healthy Human Subjects

Saqib Chowdhary, Sarah L. Nuttall, John H. Coote, Jonathan N. Townend

Abstract—Cardiac vagal control has prognostic significance in cardiac disease, but the control mechanisms of this system remain poorly understood. We have previously demonstrated a role for NO in promoting vagal control of heart rate in humans. Here we examine the influence of L-arginine, the substrate for NO synthase, on this mechanism in healthy human subjects. Eleven healthy volunteers (9 men; age, 20 to 25 years) underwent measurement of heart rate variability and baroreflex sensitivity before and during a systemic infusion of L-arginine (1 g/min; total, 30 g). To control for the fall in blood pressure, comparison was made with an infusion of the control vasodilator hydralazine. Stereospecificity of observed effects was investigated by infusion of D-arginine. Urinary nitrate and nitrite (NO\textsubscript{x}) and cGMP concentrations were measured as indexes of NO generation. L-Arginine infusion produced a drop in mean arterial pressure of 5 mm Hg. This fall in blood pressure was matched by hydralazine infusion and was not observed with either D-arginine or saline infusion. Although RR interval duration, heart rate variability, and baroreflex sensitivity all fell significantly with hydralazine, the same degree of baroreflex unloading with L-arginine produced an increase in RR interval duration and no change or even slight increases in heart rate variability and baroreflex sensitivity. In contrast, D-arginine produced falls in high-frequency indexes of heart rate variability compared with saline. Only L-arginine increased urinary NO\textsubscript{x} and cGMP excretion. In conclusion, these data demonstrate that short-term L-arginine infusion facilitates vagal control of heart rate in healthy humans, probably via increased NO synthesis. (Hypertension. 2002;39:51-56.)

Key Words: L-arginine ■ nitric oxide ■ baroreflex ■ autonomic nervous system ■ heart rate variability

Impaired autonomic regulation of the heart exerts a powerful influence on prognosis in cardiac disease. In large-scale studies, depressed markers of cardiac vagal function—heart rate variability (HRV) and baroreflex sensitivity (BRS)—have been shown to be powerfully and independently predictive of an adverse prognosis in heart failure\textsuperscript{1} and after myocardial infarction.\textsuperscript{2} However, the mechanisms controlling cardiac autonomic function in both health and cardiac disease are poorly understood. A growing body of animal evidence suggests that NO may have a significant role as a neuromodulator within this control system. NO synthase (NOS) is expressed in many localized neuronal populations, both central and peripheral, that regulate cardiovascular autonomic function. The NO generated at these sites appears to have a significant neuromodulator activity, with the net effect of cardiac vagal activation and sympathetic inhibition.\textsuperscript{3} In humans, we have previously used both systemic NOS inhibitors and exogenous NO donors to demonstrate a facilitatory role for NO in the modulation of cardiac vagal control in healthy volunteers.\textsuperscript{4} We therefore hypothesize that enhancement of NOS activity might result in a therapeutically relevant augmentation of cardiac vagal influence.

NO is synthesized by a family of NO synthases from the amino acid L-arginine, administration of which has been shown to have significant physiological effects related to enhanced NO synthesis, including improved endothelial function and vasodilatation.\textsuperscript{5-13} These effects are present despite the intracellular concentrations of L-arginine that greatly exceed those required for maximal enzyme kinetics,\textsuperscript{14} an inconsistency that has been called the arginine paradox. Although it is clear that some of the effects of L-arginine are not specific to NO, the impact of this amino acid on NO-mediated effects does appear to be of importance and constitutes a simple, clinically applicable method of enhancing NO pathway activity.

We have studied the effects of intravenous administration of L-arginine on HRV and BRS in healthy volunteers. The confounding influence of baroreflex unloading was controlled for by comparison with the effects of the non–NO-dependent vasodilator hydralazine given at a dose resulting in an equal drop in blood pressure. The specificity of effects for the NO pathway was determined by comparison with an infusion of the stereoisomer D-arginine, which shares many of the nonspecific effects of L-arginine but is not a substrate for...
TABLE 1. Baseline Hemodynamics, HRV, and BRS Before Each Drug

<table>
<thead>
<tr>
<th>Index</th>
<th>L-Arginine</th>
<th>Hydralazine</th>
<th>D-Arginine</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>85±2</td>
<td>83±2</td>
<td>87±3</td>
<td>84±2</td>
</tr>
<tr>
<td>RR interval, ms</td>
<td>932±23</td>
<td>881±38</td>
<td>955±49</td>
<td>933±44</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>75±18</td>
<td>62±5</td>
<td>72±10</td>
<td>71±9</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>71±19</td>
<td>56±8</td>
<td>66±14</td>
<td>63±11</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>47±6</td>
<td>36±7</td>
<td>42±11</td>
<td>42±9</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>4036±1163</td>
<td>2613±646</td>
<td>3456±1306</td>
<td>3510±1025</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>614±107</td>
<td>588±110</td>
<td>870±241</td>
<td>615±130</td>
</tr>
<tr>
<td>LF/HF ratio, %</td>
<td>32±13</td>
<td>32±8</td>
<td>60±36</td>
<td>50±29</td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>6233±1284</td>
<td>4128±748</td>
<td>5682±1589</td>
<td>5695±1338</td>
</tr>
<tr>
<td>α-HF, ms/mm Hg</td>
<td>21.2±2.7</td>
<td>20.0±3.0</td>
<td>22.2±5.6</td>
<td>21.3±4.3</td>
</tr>
<tr>
<td>α-LF, ms/mm Hg</td>
<td>9.6±1.1</td>
<td>10.3±1.0</td>
<td>11.1±1.7</td>
<td>9.8±1.0</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure.

NOS. Excretion of breakdown products of NO was also measured as a biochemical estimate of changes in NO pathway activity.

Methods

Subjects

Eleven healthy volunteers (9 men) 20 to 25 years of age (mean, 22 years) were studied. All subjects were normotensive (supine cuff blood pressure measurement <140/90 mm Hg at initial screening visit) with no symptoms or signs of cardiovascular disease and on no medication. Subjects were asked to abstain from food or drink for ≥2 hours before the study and from caffeine and alcohol for 24 hours. Experimental protocols were approved by our local research ethics committee, and individual written consent was obtained.

Experimental Protocol

Protocols were of a single-blind, random-order, crossover design. All subjects had a preliminary acclimatization visit when they were trained to breathe to an audio signal set close to the individual’s resting respiratory rate. Each patient was then randomly assigned to receive a 30-minute intravenous infusion of either L-arginine (1 g/min) or the control vasodilator hydralazine (loading with 20 mg/g/min) or the control vasodilator hydralazine (loading with 20 mg/2 hours before the study and from caffeine and alcohol for 24 hours. Experimental protocols were approved by our local research ethics committee, and individual written consent was obtained.

Biochemical Markers of NO Pathway Activity (Urinary Nitrate/Nitrite and cGMP Concentrations)

Combined urinary concentrations of nitrate and nitrite (NOx), stable breakdown products of NO, and cGMP were measured as surrogate markers of NO formation and activity. Urine samples were collected immediately before and after the study. Nitrate was first reduced to nitrite by enzymatic conversion with nitrate reductase, and NOx concentrations were subsequently quantified with the Griess reaction. Urinary concentrations of cGMP were assessed by ELISA (R&D systems kit assay). In each case, results are expressed as a ratio to the urinary creatinine concentration.

Statistical Analysis

Baseline hemodynamic, HRV, and BRS data were expressed as the mean of the 2 recording periods. Data for mean arterial pressure, RR interval, and urinary NOx were compared by a 2-tailed paired Student’s t test. Differences between groups for indexes of HRV and BRS were determined by use of the Wilcoxon signed-rank test for paired data and the Mann-Whitney test for unpaired data. Statistical significance was taken as P<0.05, and values are expressed as mean±SE.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

The frequency of metronomic breathing was within the range 0.18 to 0.23 Hz. Baseline values for mean arterial pressure,
RR interval, and indexes of HRV and BRS were not significantly different between the groups before administration of L-arginine, hydralazine, d-arginine, or saline (Table 1). Infusion of L-arginine and hydralazine both resulted in closely matched and statistically significant falls in mean arterial pressure (the primary aim with respect to hemodynamic matching) mainly because of a drop in diastolic pressure (Figure 1). This fall in blood pressure was apparent after 10 minutes of L-arginine infusion (ie, after 10 g). There was little additional decrease observed after 30 minutes (ie, after 30 g). The blood pressure response to hydralazine closely matched that seen with L-arginine at 10 minutes (ie, immediately after the 20-mg loading dose) with matching maintained to the 30-minute time period with respect to diastolic and mean blood pressures, although systolic and pulse pressures rose slightly more with hydralazine. Neither saline nor D-arginine altered any measure of blood pressure (Figure 1).

In contrast to the matched blood pressure responses, the changes in RR interval during infusion of L-arginine and the control vasodilator agent hydralazine were discrepant. L-Arginine resulted in a reduction in heart rate, causing a statistically significant increase in mean RR interval length, whereas hydralazine produced cardioacceleration, considerably shortening the RR interval (Table 2).

Changes in HRV and BRS were also dissimilar between the 2 vasodilators. Despite baroreflex unloading, indexes of overall HRV (SDNN and total power) and vagally determined HF indexes of HRV (RMSSD, pNN50, and HF power) were unchanged during L-arginine infusion. In contrast, there were substantial reductions in all these indexes with hydralazine. Similarly, the ratio of LF to HF was unchanged with L-arginine, which is in contrast to the significant rise seen with hydralazine. As a further index of vagal cardiac regulation, cross-spectral BRS (both α-HF and α-LF) also showed significant falls with hydralazine but no change or even slight rises with L-arginine (Table 2).

Saline had no significant effect on HRV or BRS. Compared with saline infusion, d-arginine had no effect on most of the autonomic indexes but resulted in a small but significant reduction in measures of HF HRV, namely RMSSD and HF power, as well as in α-HF (Table 3).

Measures of whole-body NO pathway activity (urinary excretion of both NOx and cGMP) were significantly increased by L-arginine infusion but not by hydralazine, saline, or d-arginine (Figure 2).

**Discussion**

The primary finding of this study is that despite a fall in blood pressure, L-arginine infusion resulted in no change or even slight rises in measures of HRV and BRS that correlate with cardiac vagal control. In contrast, a similar reduction in
arterial pressure produced by the control vasodilator hydralazine caused baroreflex-mediated attenuation of these indexes. These data demonstrate that L-arginine exerts a stimulatory effect on cardiac vagal control, resulting in its preservation during baroreflex unloading. Although L-arginine exhibits a number of biological actions, the results of our previous investigations, in which we have demonstrated a vagotonic action of both endogenous NO generation and exogenous NO donors, lead us to hypothesize that the autonomic actions of L-arginine observed in this study were mediated by enhanced NO synthesis. Several lines of evidence from the present study support this conclusion.

First, the effects of L-arginine on blood pressure, heart rate, HRV, and BRS seen with D-arginine may have been hormonal effects as L-arginine but does not act as a substrate for NOS, this strongly suggests specificity for NO-mediated actions. We hypothesize that the small falls in vagally mediated HRV and BRS seen with L-arginine, together with specific NO-mediated vasodilatation, the normal reflex vagal withdrawal appears to have been overridden by the vagotonic effects of increased NO synthesis. Finally, in agreement with previous studies, the urinary excretion of NO, over the time course of the experiment was increased by L-arginine but not by D-arginine, saline, or hydralazine, providing further evidence of a stereospecific increase in NO generation.

The concept that the L-arginine supply can be rate limiting to NO synthesis is controversial because biochemical considerations dictate that intracellular concentrations of L-arginine greatly exceed those required for maximal NOS activity.

### TABLE 2. Change in RR Interval, HRV, and BRS Observed With Each Vasodilator Drug

<table>
<thead>
<tr>
<th>Index</th>
<th>L-Arginine for 10 min</th>
<th>Hydralazine for 10 min</th>
<th>L-Arginine for 30 min</th>
<th>Hydralazine for 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR interval, ms</td>
<td>41±16§</td>
<td>-236±26∫</td>
<td>22±18∫</td>
<td>-245±18∫</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>-3±4†</td>
<td>-35±5¶</td>
<td>1±4‡</td>
<td>-36±5¶</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>-1±5‡</td>
<td>-36±6¶</td>
<td>0±5‡</td>
<td>-37±6¶</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>-2±3¶</td>
<td>-28±6∫</td>
<td>-1±3¶</td>
<td>-28±6∫</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>67±591†</td>
<td>-2069±617</td>
<td></td>
<td>210±556†</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>146±237†</td>
<td>-427±99¶</td>
<td>64±102*</td>
<td>-417±101¶</td>
</tr>
<tr>
<td>LF/HF ratio, %</td>
<td>-3±7*</td>
<td>52±29∥</td>
<td>-8±10†</td>
<td>130±85§</td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>101±702†</td>
<td>-3106±659¶</td>
<td>289±721†</td>
<td>-3215±681¶</td>
</tr>
<tr>
<td>α-HF, ms/mm Hg</td>
<td>2.3±0.6¶</td>
<td>-11.9±2.6∥</td>
<td>-0.3±1.3¶</td>
<td>-12.1±2.7∥</td>
</tr>
<tr>
<td>α-LF, ms/mm Hg</td>
<td>1.0±0.7†</td>
<td>-5.0±1.3∥</td>
<td>4.1±0.9∥</td>
<td>-4.7±1.3∥</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01, ‡P<0.001, change with L-arginine vs change with hydralazine at the same time point.
§P<0.05, ||P<0.01, ¶P<0.001 for significance of change from baseline.

### TABLE 3. Change in RR Interval, HRV, and BRS Observed For Nonvasodilator Control Drugs: 10-Minute Data

<table>
<thead>
<tr>
<th>Index</th>
<th>D-Arginine</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR interval, ms</td>
<td>13±10</td>
<td>20±9</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>-6±6</td>
<td>3±3</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>-8±4*</td>
<td>6±4</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>-3±2</td>
<td>2±3</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>-869±481*</td>
<td>558±396</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>-303±216</td>
<td>-15±107</td>
</tr>
<tr>
<td>LF/HF ratio, %</td>
<td>-15±28</td>
<td>-9±7</td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>-999±890</td>
<td>480±644</td>
</tr>
<tr>
<td>α-HF, ms/mm Hg</td>
<td>-3.0±1.8*</td>
<td>1.2±1.1</td>
</tr>
<tr>
<td>α-LF, ms/mm Hg</td>
<td>-2.5±1.5</td>
<td>0.6±1.4</td>
</tr>
</tbody>
</table>

*P<0.05 for change with d-arginine vs change with saline.
Although some studies have failed to show a significant vasomotor influence of systemic L-arginine infusion in healthy volunteers, these are outnumbered by the studies that do show falls in blood pressure and total peripheral resistance in healthy volunteers and hypertensive subjects. Furthermore, L-arginine administration has been shown consistently to improve endothelial NO-mediated vasodilator responses in patients with hypercholesterolemia\(^5\) and in the atheromatous coronary circulation.\(^9\) In agreement with our data, many healthy volunteer studies have demonstrated hemodynamic actions after only 10 g of L-arginine (reducing the likelihood of nonstereospecific actions) and, importantly, showed biochemical evidence of increased NO production. One possible answer to this arginine paradox may lie in the intracellular compartmentalization of constitutive NOS within caveoleae, which, in conjunction with certain colocalized arginine transporters (eg, cationic amino acid transporter-1), may serve to regulate the supply of L-arginine to NOS.\(^20\)

Few other studies have examined the influence of L-arginine on HRV in humans. Nomura et al\(^21\) infused 30 g of L-arginine (1 g/min) into 8 healthy volunteers and found significant increases in indexes of vagal cardiac control at as early as 5 to 10 minutes of infusion despite a fall in systolic blood pressure. Thus, the vagotonic effects of L-arginine in their study were even more pronounced than those observed in our population, a difference perhaps explained by ethnic variation. These investigators did not directly study the specificity of autonomic effects to the L-arginine–NO pathway. Instead, they indirectly concluded that the autonomic influence of L-arginine was independent of NO, despite a rise in L-citrulline, a byproduct of NO production, because it was not reproduced by infusion of the NO donor isosorbide dinitrate. We suggest that the vagotonic effects of isosorbide dinitrate in the study of Nomura et al may have been blunted by a greater degree of baroreflex unloading and the observed activation of humoral factors such as noradrenaline, renin, and angiotensin, effects not seen with L-arginine. We have previously demonstrated that when the fall in blood pressure produced by an NO donor (sodium nitroprusside) is matched with that resulting from a non–NO-dependent vasodilator (hydralazine), levels of vagally mediated HRV are higher with the NO donor.\(^5\) L-Arginine has also been shown to increase HF power in patients with liver cirrhosis.\(^22\) Our protocol cannot localize the site of action of L-arginine in its modification of cardiac autonomic activity, but animal data suggest that L-arginine may act as a substrate in a number of central and peripheral neuronal populations within the cardiac baroreflex arc that exhibit a discrete localization of neuronal NOS.\(^3\) Functional activity for L-arginine has been demonstrated within the primary relay for baroreceptor afferents in the brainstem, the nucleus tractus solitarii. Microinjection of L-arginine but not D-arginine into the nucleus tractus solitarii of rats caused an increase in nucleus tractus solitarii neuronal activity, leading to a fall in systemic blood pressure and bradycardia. Both the neuronal and hemodynamic effects were blocked by a neuronal NOS inhibitor and an NO scavenger and reproduced by NO donors.\(^23,24\) Furthermore, NO has been shown to increase neuronal activity within other central sites regulating parasympathetic outflow to the heart.\(^25\) Peripherally, NO potentiates the bradycardic effects of parasympathetic stimulation\(^26–28\) and enhances the ability of the efferent vagus to antagonize sympathetic cardiac responses,\(^29\) with both presynaptic and postsynaptic mechanisms postulated.\(^26,28\)

**Study Limitations**

We cannot exclude a sympathetic nervous influence of L-arginine on our results. Although the ratio of LF to HF and α-LF contain some information on sympathetic cardiac control, they lack specificity because they are also influenced by vagal activity.\(^15\) L-Arginine may have acted to inhibit sympathetic nervous activity either through the sympatholytic actions of NO or via stimulation of central α-receptors by its metabolite agmatine.\(^30\) However, L-arginine infusion has been shown not to alter levels of noradrenaline spillover in healthy human subjects.\(^21\)

Establishing the specificity of observed effects to activation of NOS may have been aided by coadministration of NOS inhibitors. However, opposing effects would have extended to hemodynamic and autonomic influence, rendering the final results difficult to interpret. Finally, we did not study the influence of other cofactors for NOS activity such as tetrahydrobiopterin. The supply of L-arginine relative to BH4 may be critical in determining whether NOS generates NO or O2•−, and it is possible that the observed autonomic activity of L-arginine may have been even greater if coadministered with tetrahydrobiopterin.

In conclusion, we have demonstrated that a vasodepressor dose of L-arginine did not result in heart rate acceleration or depression of vagally mediated autonomic indexes, in contrast to a control vasodilator. These findings were not reproduced by D-arginine, which, in conjunction with biochemical evidence of increased NO generation, suggests that these effects were mediated by the L-arginine–NO pathway. Extensive study has been made of the effects of L-arginine supplementation to correct endothelial dysfunction observed in various cardiac disease states. Our results now suggest that the potential for L-arginine therapy to correct the impairment of vagal control associated with mortality in cardiac disease also merits investigation.

**Acknowledgments**

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**References**


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