Relationship between muscle sympathetic nerve activity and systemic hemodynamics during nitric oxide synthase inhibition in humans

N. Charkoudian,1,2,3 M. J. Joyner,1,2,3 S. A. Barnes,1,2 C. P. Johnson,2 J. H. Eisenach,2,3 N. M. Dietz,2,3 and B. G. Wallin4

1Department of Physiology and Biomedical Engineering, 2Department of Anesthesiology and Critical Care Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota; and 3Institute of Clinical Neurosciences, Göteborg University, Göteborg, Sweden

Submitted 7 March 2006; accepted in final form 20 April 2006

Charkoudian, N., M. J. Joyner, S. A. Barnes, C. P. Johnson, J. H. Eisenach, N. M. Dietz, and B. G. Wallin. Relationship between muscle sympathetic nerve activity and systemic hemodynamics during nitric oxide synthase inhibition in humans. Am J Physiol Heart Circ Physiol 291: H1378–H1383, 2006. First published April 28, 2006; doi:10.1152/ajpheart.00234.2006.—Large interindividual differences exist in resting sympathetic nerve activity (SNA) among normotensive humans with similar arterial pressure (AP). We recently showed inverse relationships of resting SNA with cardiac output (CO) and vascular adrenergic responsiveness that appear to balance the influence of differences in SNA on blood pressure. In the present study, we tested whether nitric oxide (NO)-mediated vasodilation has a role in this balance by evaluating hemodynamic responses to systemic NO synthase (NOS) inhibition in individuals with low and high resting muscle SNA (MSNA). We measured MSNA via peroneal microneurography, CO via acetylene uptake and AP directly, at baseline and during increasing systemic doses of the NOS inhibitor L-NMMA. Baseline MSNA ranged from 9 to 38 bursts/min (13 to 68 bursts/100 heartbeats). L-NMMA caused dose-dependent increases in AP and total peripheral resistance and reflex decreases in CO and MSNA. Increases in AP with L-NMMA were greater in individuals with high baseline MSNA (PANOVA < 0.05). For example, after 8.5 mg/kg of L-NMMA, in the low MSNA subgroup (n = 6, 28 ± 4 bursts/100 heartbeats), AP increased 9 ± 1 mmHg, whereas in the high-MSNA subgroup (n = 6, 58 ± 3 bursts/100 heartbeats), AP increased 15 ± 2 mmHg (P < 0.01). The high-MSNA subgroup had lower baseline CO and smaller decreases in CO with L-NMMA, but changes in total peripheral resistance were not different between groups. We conclude that differences in CO among individuals with varying sympathetic traffic have important hemodynamic implications during disruption of NO-mediated vasodilation.

HUMAN MUSCLE SYMPATHETIC NERVE activity (MSNA) is composed of vasoconstrictor impulses grouped in pulse synchronous bursts. At rest, normotensive subjects with similar blood pressures display large, reproducible interindividual differences in the number of bursts (14, 15). This interindividual variability appears to be consistent among muscle, cardiac, and renal vascular beds when sympathetic activity to the latter two vascular beds is measured by using norepinephrine spillover techniques; MSNA also correlates with whole body norepinephrine spillover (18, 19). There is not, however, a consistent relationship among individuals between resting MSNA and resting blood pressure (3, 14, 15). This contrasts with the presence of marked short-term baroreflex regulation of MSNA in individual subjects: on a beat-to-beat basis, even minor changes in arterial pressure elicit robust reflex responses in MSNA, irrespective of whether the subject has few or many bursts (15). This apparent paradox suggests the existence of other long-term modifiers of the interaction between arterial pressure and MSNA.

We recently showed that cardiac output (CO) may be such a modifier: CO at rest was inversely related to resting MSNA and to baroreflex control of MSNA at rest (3). Additionally, peripheral vascular responsiveness to adrenergic stimulation was inversely related to resting MSNA (4), suggesting that both of these factors have important roles in the integrated balance that maintains normal, and similar, blood pressures among individuals with widely varying resting MSNA. With regard to vascular function, Skarphedinsson et al. (14) previously reported that circulating nitrate levels [a plasma marker of nitric oxide (NO) bioactivity] were directly correlated with MSNA, suggesting that NO-mediated vasodilation may also be a factor in this balance.

In the present study, we wished to further evaluate a potential role of NO-mediated vasodilation in balancing the influence of variable levels of resting SNA among normotensive individuals. To do this, we measured hemodynamic responses to progressive, systemic inhibition of NO synthase (NOS) with Nω-monomethyl-l-arginine (l-NMMA). We reasoned that the changes in arterial pressure and in total peripheral resistance (TPR) with l-NMMA would represent the contribution of NO to these variables at baseline (9). We hypothesized that individuals with higher resting MSNA would exhibit larger increases in arterial pressure and TPR with NOS inhibition and that individuals with lower resting MSNA would exhibit smaller changes. This would represent greater NO-mediated vasodilation at baseline in the former group, which could be one of the mechanisms responsible for keeping blood pressure normal in the face of higher sympathetic vasoconstrictor tone.

METHODS

Subjects

The protocol for this study was approved by the Institutional Review Board of the Mayo Foundation. Eighteen healthy young men [age, 26.9 ± 1.2 (SE) yr; height, 1.78 ± 0.03 m; body wt, 77.6 ± 2.5 lb] were studied.
kg] volunteered to participate and gave written informed consent. Subjects were nonsmokers with no history of cardiovascular or other chronic diseases. They were asked not to consume anything except water within 2 h before the experiment and not to consume caffeine the day of the experiment or alcohol within 24 h of the experiment.

**Measurements**

All studies were performed in a General Clinical Research Center laboratory at the Mayo Clinic, where ambient temperature was controlled between 22° and 24°C. On arrival at the laboratory, subjects rested quietly in the supine position during instrumentation. A 5-cm, 20-gauge catheter was placed in the radial artery of the nondonominant arm with the use of aseptic technique after local anesthesia with 2% lidocaine. This catheter was connected to a pressure transducer placed at heart level and used for measurement of arterial pressure. On the contralateral arm, an 18-gauge intravenous catheter was placed for systemic delivery of L-NMMA. A three-lead ECG was used for continuous monitoring of heart rate (HR).

CO was measured by using the open-circuit acetylene uptake technique, as previously described (11). This technique has been validated against direct Fick measurements of CO for a range of CO values (11). The instrumentation period included a practice measurement of CO to familiarize the subject with the procedure. This practice value was not included in the CO data presented in the results.

Multunit MSNA was recorded with a tungsten microelectrode in the peroneal nerve, posterior to the fibular head, as described by Sundlöf and Wallin (16). The recorded signal was amplified 80,000-fold, band-pass filtered (700 to 2,000 Hz), rectified, and integrated (resistance-capacitance integrator circuit, time constant 0.1 s) by a nerve-traffic analyzer.

**Protocol**

Subjects rested supine throughout the protocol, which is shown in Fig. 1. We collected data at baseline and during 20-min intravenous infusions of each of four doses of L-NMMA: 0.05, 0.125, 0.25 and 0.50 mg·kg⁻¹·min⁻¹. Because L-NMMA has a long biological half-life (13), the doses were cumulative, such that total doses were 1, 3.5, 8.5, and 18.5 mg/kg, respectively.

Throughout the protocol, we continuously recorded arterial pressure, ECG, and integrated MSNA. CO was measured in duplicate at the end of the baseline period (measurements separated by ~5 min) and during each dose of L-NMMA. To measure values of CO during time periods that were approximately at steady state for each dose of L-NMMA, CO was measured once during the 18th minute of each dose and once during the 3rd minute of the subsequent dose (at which time the subsequent dose of L-NMMA had not yet had a significant effect). These two measurements were averaged as the CO value for that dose.

**Data Analysis**

Data were sampled at 240 Hz and stored on a personal computer for offline analysis. MSNA, HR, and mean arterial pressure (MAP) were taken as 4-min averages during the 4 min immediately preceding each CO measurement. Stroke volume (SV) was calculated as CO/HR; TPR was calculated as MAP/CO. Sympathetic bursts in the integrated neurogram were identified by a custom-manufactured automated analysis program; burst identification was then corrected by visual inspection by a single investigator. MSNA was expressed as burst incidence (in bursts/100 heart beats) and burst frequency (in bursts/min).

**Subgroup analysis.** The main goal of the present study was to assess whether resting MSNA was associated with differences in hemodynamic responses to NOS inhibition. Therefore, subjects were divided into three subgroups on the basis of low (n = 6), medium (n = 6), and high (n = 6) baseline MSNA. To maximize our ability to detect differences based on resting MSNA, we then compared the low- and high-MSNA subgroups in terms of responses to L-NMMA.

**Statistical analysis.** Group average data are expressed as means ± SE. One-way repeated-measures ANOVA was used to analyze the influence of L-NMMA on group mean data for all cardiovascular variables and MSNA. Two-way ANOVA (repeated measures on dose of L-NMMA) was used to assess whether there were differences between low- and high-MSNA subgroups in terms of responses to L-NMMA. Statistical significance was set at P < 0.05.

**RESULTS**

**Overall Group Average Responses**

Table 1 shows average data at baseline for all subjects. Figure 2 shows group average hemodynamic responses to progressive systemic doses of L-NMMA. As expected, during L-NMMA, MAP increased (Fig. 2A), CO decreased (Fig. 2B), and TPR increased (Fig. 2C) in a dose-dependent manner. The pressor effect of the L-NMMA caused dose-dependent reflex reductions in MSNA (Fig. 3). Changes in MSNA were similar whether expressed as bursts per minute or bursts per 100 heartbeats. HR also exhibited dose-dependent reflex reductions during L-NMMA, falling from 58 ± 2 beats/min at baseline to 49 ± 2 beats/min at the highest dose of L-NMMA.

**Subgroup Analysis**

Baseline characteristics of subjects in low- and high-MSNA subgroups are shown in Table 2. MAP was not different between groups. Subjects with low MSNA had significantly higher CO and HR and significantly lower TPR than those in the high-MSNA subgroup. These differences are consistent with our previous report (3) of an inverse relationship between CO and MSNA and a direct relationship between MSNA and TPR. We subsequently compared these two subgroups to evaluate the influence of resting MSNA on hemodynamic responses to NOS inhibition. The corresponding characteristics

---

**Table 1. Group average data for neural and cardiovascular variables at baseline**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MSNA, bursts/min</th>
<th>24±2</th>
<th>MSNA, bursts/100 heartbeats</th>
<th>44±3</th>
<th>MAP, mmHg</th>
<th>95±2</th>
<th>CO, l/min</th>
<th>6.1±0.4</th>
<th>TPR, mmHg·l⁻¹·min⁻¹</th>
<th>16.28±0.98</th>
<th>HR, beats/min</th>
<th>57±2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are means ± SE for n subjects. MSNA, muscle sympathetic nerve activity; MAP, mean arterial blood pressure; CO, cardiac output; TPR, total peripheral resistance; HR, heart rate.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
for the middle subgroup \((n = 6)\) were as follows: resting MSNA, 45 \pm 2 bursts/100 heartbeats; MAP, 93 \pm 3 mmHg; CO, 5.45 \pm 0.46 l/min; TPR, 17.8 \pm 1.9 mmHg\(\cdot\)l\(-1\)\(\cdot\)min; HR, 55 \pm 5 beats/min.

Table 2. Average data for low- and high-MSNA subgroups

<table>
<thead>
<tr>
<th></th>
<th>Low MSNA</th>
<th>High MSNA</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>MSNA, bursts/100 heartbeats</td>
<td>28(\pm)4</td>
<td>58(\pm)3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>94(\pm)3</td>
<td>96(\pm)4</td>
<td>0.72</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>7.17(\pm)0.53</td>
<td>5.60(\pm)0.49</td>
<td>0.02</td>
</tr>
<tr>
<td>TPR, mmHg(\cdot)l(-1)(\cdot)min</td>
<td>13.41(\pm)0.91</td>
<td>17.88(\pm)1.90</td>
<td>0.04</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>64(\pm)3</td>
<td>51(\pm)2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE for \(n\) subjects.

Figure 4 shows hemodynamic responses during t-NMMA in the low- and high-MSNA subgroups. The high-MSNA subgroup had a significantly greater MAP response to t-NMMA compared with the low-MSNA subgroup (Fig. 4A; main effect of group: \(P_{\text{ANOVA}} < 0.05\)). Conversely, the changes in CO during t-NMMA were smaller for the high-MSNA subgroup, probably due to the lower baseline CO values in this group (Fig. 4B; main effect of group: \(P_{\text{ANOVA}} < 0.05\)). Although initial CO was higher in the low-MSNA group, final absolute values for CO at the highest dose of t-NMMA were not different between subgroups (high-MSNA subgroup: 4.09 \pm 0.46 vs. low-MSNA subgroup: 4.52 \pm 0.50 l/min, \(P > 0.05\)). The decrease in HR with t-NMMA tended to be greater in...
subjects with low resting MSNA, consistent with the greater decrease in CO; however, this trend did not reach statistical significance ($P_{\text{ANOVA}} = 0.07$ for main effect of subgroup). The greatest differences between the two subgroups were seen at the two highest doses of 1-NMMA (0.25 and 0.50 mg·kg$^{-1}$·min$^{-1}$), where ΔHR in the low-MSNA subgroup averaged −9 ± 1 and −11 ± 2 beats/min, respectively, and ΔHR in the high-MSNA subgroup was −5 ± 1 and −6 ± 1 beats/min, respectively. Although not statistically significant between subgroups, these differences in ΔHR were probably the main contributors to the differences in ΔCO seen between groups. Changes in SV were not different between groups ($P_{\text{ANOVA}} > 0.5$); for example, at 0.25 and 0.50 mg·kg$^{-1}$·min$^{-1}$ 1-NMMA, ΔSV values were −24 ± 5 and −25 ± 5 ml, respectively, in the low-MSNA subgroup, and −19 ± 4 and −18 ± 4, respectively, in the high-MSNA subgroup.

The changes in TPR during 1-NMMA were not different between subgroups, as shown in Fig. 4C. These changes were also not different if expressed as total vascular conductance ($P > 0.05$; data not shown). The reflex reduction of MSNA during 1-NMMA was also not different between subgroups, as shown in Fig. 5.

**DISCUSSION**

We measured hemodynamic responses to progressive, systemic NOS inhibition with 1-NMMA to quantify the contribution of systemic NO-mediated vasodilation to arterial pressure and TPR in subjects with varying baseline MSNA. Our major new finding is that individuals with higher baseline MSNA exhibited greater increases in MAP during progressive NOS inhibition than did those with low MSNA. However, in contrast to our hypothesis, this pressor response did not involve a greater increase in TPR in the high-MSNA subgroup. The main hemodynamic basis for the larger pressor response in the high-MSNA subgroup appeared to be a smaller reduction in CO in individuals with high baseline MSNA, who had lower CO at baseline. Importantly, there were no significant differences between groups in the reflex changes of MSNA during NOS inhibition, suggesting that differences in baroreflex control of the peripheral circulation were not major contributors to the different pressor responses between groups. With regard to this last point, it does not appear that systemic 1-NMMA per se alters baroreflex control of MSNA in humans (5, 10).

The importance of CO in determining differential hemodynamic responses to NOS inhibition is consistent with our recent finding of a balance between CO and MSNA among normotensive humans at rest: individuals with lower MSNA had higher CO and vice versa (3). The present results indicate that this relationship has implications for arterial pressure regulation during disruptions of NO-mediated vasodilation as well. The fact that the high-MSNA subgroup had significantly lower resting CO than did the low-MSNA subgroup meant that they had less "reserve" of CO to decrease and buffer the pressor response to NOS inhibition. Although we cannot conclusively state which CO-related factor is most important for the differential effects induced by the NOS inhibition, changes in HR demonstrated a tendency to be greater in subjects with low baseline MSNA ($P = 0.07$), suggesting that changes in HR may have been quantitatively more important than changes in SV in this regard.

Previous findings of Skarphedinsson et al. (14) suggested that NO-mediated vasodilation might balance sympathetically mediated vasoconstriction in individuals with higher resting MSNA under baseline conditions. Data from the present study suggest that the extent of systemic NO-mediated vasodilator "tone" is not consistently different between individuals with lower and higher MSNA. However, more specific conclusions about the role of NO may be difficult to reach due to the complexity of the regulation of NO-mediated vasodilation and the fact that its quantitative importance differs among vascular beds (12).

With regard to potential differences in control of vascular resistance between individuals with low and high resting MSNA, it appears that differences in mechanisms of adrenergic vasoconstriction contribute to the balance of factors that maintain normal blood pressures in the face of widely varying chronic levels of SNA. In our recent study, we reported an inverse relationship between resting MSNA and vasoconstrictor responses to adrenergic stimulation with norepinephrine and tyramine (4). This suggests that "buffered" vasoconstrictor responses to sympathetic stimuli contribute to the integrated mechanism by which normotensive individuals with higher nerve activity do not have higher blood pressure. With regard to interpretation of our present data, it has been shown that adrenergic vasoconstriction can be modified by NO (7, 8). Exogenous NO attenuated adrenergic vasoconstriction in human skin (8), and combined blockade of NO and prostaglandins (but not either blockade individually) augmented adrenergic vasoconstriction in exercising human skeletal muscle (7). It is not clear whether those previous findings indicate that our present systemic NOS inhibition had a generalized effect to alter responsiveness to SNA in our resting subjects. However, in light of our previous report regarding the role of adrenergic responsiveness in the overall balance of factors affecting blood pressure, it is important to consider this possible modifier in the interpretation of our present results.

Our present findings during experimental NOS inhibition may have important clinical and pathophysiological implications for conditions in which NO vasodilation pathways are impaired. Data from the present study and from previous work (3, 4) suggest that MSNA, CO, and vascular adrenergic responsiveness coexist in a balance that is important for normal arterial pressure regulation. We report here that even small impairments of NO-mediated vasodilation (seen in the present

Fig. 5. Subgroup analysis of MSNA responses during increasing doses of 1-NMMA. Despite differences in baseline MSNA between the two groups, reductions in MSNA during 1-NMMA were not different between groups.
study as lower doses of l-NMMA) resulted in a greater disruption of this balance in individuals with higher MSNA than in those with lower MSNA. If this evidence is extrapolated to pathophysiological conditions in which NO-mediated vasodilation is impaired [such as in patients with hyperlipidemia (1, 2) and in normotensive offspring of patients with essential hypertension (17)], individuals with higher MSNA may be at greater risk for development of hypertension than individuals with similar impairment of NO function who have lower baseline MSNA. An important caveat to this point is that we do not know how other pathophysiological aspects of these complex conditions may affect the balance of factors we have identified.

Potential Limitations

One of the challenges involved in studying the role of NO in cardiovascular control is its very short biological half-life. NO is rapidly converted to nitrite/nitrate (NOx), making interpretable measurements of NO in intact systems problematic. Therefore, studies evaluating the role of NO have used indirect indices of its activity, such as measurement of NOx (14) and/or measurement of variables of interest before and after inhibition of the NOS enzyme (6, 9). The rationale behind this latter approach (used in the present study) is that the extent of change in a given variable with NOS inhibition will represent the contribution of NO to that variable at baseline. A limitation to the use of systemic NOS inhibition is its pressor effect; this results in reflex adjustments to changes in pressure that may confound interpretation of results.

In the present study, we were specifically interested in the changes in blood pressure induced by NOS inhibition. Furthermore, it does not appear from the literature that systemic NOS inhibition in humans alters baroreflex control per se (5, 9, 10). In animal models, NO appears to have a role in central inhibition of sympathetic efferent activity (20). However, previous studies (5, 9, 10) using systemic l-NMMA in humans have shown no evidence of central effects. Cui et al. (5) reported that systemic infusions of l-NMMA did not alter baroreflex control of MSNA during head-up tilt. Although those authors observed a reflex decrease in MSNA due to the pressor effect of systemic NOS inhibition (similar to that seen in the present study), they saw no change in the sympathoexcitatory response to head-up tilt. Similarly, Hansen et al. (10) concluded that systemic l-NMMA in humans elicited reflex changes in MSNA that were explained entirely by the hemodynamic effects of NOS inhibition, with no evidence for central effects on sympathetic outflow.

When microneurography is used in humans, the number of bursts (expressed as bursts/min or bursts/100 heartbeats) is very reproducible in a given individual and therefore appropriate for comparison among individuals. Burst size, on the other hand, varies based on the strength of the burst (i.e., the number of individual action potentials in a given burst) as well as on the proximity of the electrode to the nerve itself. This latter factor varies in an unknown way among individuals, making it more problematic to calculate “total activity” (the number of bursts multiplied by the total area of all bursts) among or between individuals. In the present study, we used burst number as the most reproducible way to quantify nerve activity, and therefore to classify individuals as having low, medium, or high activity. Had we been able to reliably compare total activity among subjects, this might have given us a more comprehensive picture of resting MSNA and therefore more power to detect the differences in hemodynamic responses to l-NMMA as functions of resting MSNA.

In summary, systemic NOS inhibition caused greater increases in arterial pressure in individuals with high baseline MSNA compared with those with low baseline MSNA. The effect was due primarily to smaller reductions in CO in the subjects with high baseline MSNA, who also had lower CO values at baseline. We conclude that differential regulation of CO among individuals with different baseline MSNA resulted in different pressor responses to NOS inhibition. These neural-hemodynamic interactions may have important implications for the development of hypertension in conditions in which one or more elements of the NO vasodilation pathway are compromised.

ACKNOWLEDGMENTS

We are grateful to the subjects for their enthusiastic participation and to Shelly Roberts, Diane Wick, Karen Krucker, Ruth Kraft, and Tomas Karlsson for excellent assistance in the conduct of these studies and analysis of data.

GRANTS

These studies were supported by National Institutes of Health Grants HL-73884, NS-32352, and RR-00585 and by Swedish Medical Research Council Grant 12170.

REFERENCES


AJP-Heart Circ Physiol • VOL 291 • SEPTEMBER 2006 • www.ajpheart.org


