Plasma Norepinephrine in the Evaluation of Baroreceptor Function in Humans

Steven H. Grossman, M.D., Dwight Davis, M.D., J. Cauley Gunnelles, M.D., and David G. Shand, M.B., Ph.D.

SUMMARY The value of plasma norepinephrine measurement in assessing baroreceptor-mediated changes in sympathetic vasomotor activity was studied in seven healthy normotensive volunteers. Blood pressure was decreased by graded steady-state infusions of sodium nitroprusside (25–100 μg/min) and increased by infusions of phenylephrine (25–100 μg/min) at rates producing a 10% to 20% change in diastolic blood pressure. Sodium nitroprusside produced significant decreases in diastolic blood pressure (p < 0.01) and calculated mean arterial blood pressure (p < 0.005), and increases in heart rate (p < 0.001) and plasma norepinephrine (p < 0.001). Phenylephrine administration produced increases in systolic (p < 0.005), diastolic (p < 0.005), and mean blood pressure (p < 0.001). Heart rate (p < 0.001) and plasma norepinephrine (p < 0.05) fell. The absolute changes in diastolic and mean pressure and heart rate were not significantly different for the two drugs, but were of opposite sign; however, the increase in plasma norepinephrine during hypotension was greater than the decrease during hypertension (p = 0.02). We conclude that plasma norepinephrine changes appropriately in response to altered blood pressure and that the response is greater to a given fall than to a rise in blood pressure, consistent with known changes in sympathetic vasomotor outflow.

(Hypertension 4:566–571, 1982)

KEY WORDS • sympathetic vasomotor outflow • catecholamines • baroreflexes

The study of baroreflex function in humans has been hampered by the inability to study selectively the various limbs of the reflex arc. The heart rate response to induced blood pressure changes has been the main test of baroreceptor function but involves largely vagal efferents.1–6 Abnormalities of this reflex have suggested that the baroreceptors are "reset" in human hypertension,7–11 but since the major hemodynamic abnormality in stable hypertension in man is elevated peripheral vascular resistance,12 a measure of the sympathetic nervous system response would be more important.

The present study describes a relatively simple and noninvasive test of baroreceptor-mediated changes in sympathetic vasomotor outflow as assessed by the plasma norepinephrine response to nitroprusside-induced hypotension and phenylephrine-induced hypertension in a group of normotensive volunteers.

Methods

Seven healthy volunteers (four women, three men, aged 25–40 years) were studied using a protocol approved by the Duke University Human Research Advisory Committee. On the morning of the study a venous cannula was placed in each arm, the limb leads of a standard electrocardiograph were attached, and a blood pressure cuff was applied. Two of the investigators were present throughout the study, and quiet conversation was ongoing. No subject slept.

After at least 30 minutes, blood pressure and heart rate recording was begun at 1-minute intervals. Blood pressure was determined indirectly either by Arteriosonde (Roche) or Blood Pressure Indicator 2200 (Parke-Davis). Heart rate was determined from the electrocardiogram (ECG) over a 30- to 45-second interval.

At the end of a 10-minute control period, blood was withdrawn from the intravenous line ipsilateral to the sphygmomanometer for plasma norepinephrine determination. A controlled infusion of sodium nitroprusside or phenylephrine was begun at a rate of 25 μg/min, and 4 ml blood samples for norepinephrine were taken after 10 minutes. The procedure was repeated at rates of 50, 75, and 100 μg/min sequentially, or until the dose was reached that produced a 10% to 20% change in diastolic blood pressure. The infusion was discontinued, and after at least 20 minutes when vital signs had returned to baseline, the other drug (either

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TABLE 1. Heart Rate, Blood Pressure, and Plasma Norepinephrine Response to Graded Infusion of Nitroprusside and Phenylephrine

<table>
<thead>
<tr>
<th>Subject</th>
<th>Heart rate (beats/min)</th>
<th>Blood pressure (mm Hg)</th>
<th>Norepinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NP,1</td>
<td>NP,2</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>75</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>86</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>85</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>76</td>
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</tr>
<tr>
<td>6</td>
<td>70</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>84</td>
<td>88</td>
</tr>
</tbody>
</table>

C = control period. NP,1; NP,2; P,1 and P,2 = intermediate and maximum nitroprusside and phenylephrine doses, respectively.

Nitroprusside or phenylephrine) was infused under an identical protocol. The subjects were observed until vital signs had returned to baseline.

Blood samples were collected in chilled syringes and transferred to chilled stoppered tubes containing a solution of ethyleneglycol-tetraacetic acid (EGTA) and reduced glutathione then centrifuged at 4° to 10°C and the plasma stored at −20°C until assayed. Plasma norepinephrine was measured radioenzymatically by the method of Peuler and Johnson.13 Internal standards for each sample and a control sample were used with each day’s determination. On one assay run, aliquots of the phenylephrine, 100 μg/ml, and nitroprusside solutions, 100 μg/ml, were assayed directly and after addition to a control plasma sample. No interference with the norepinephrine assay was observed.

The data were analyzed by Wilcoxon’s Signed Rank Test or Friedman’s nonparametric analysis of variance and nonparametric multiple comparison when applicable.

Results

Heart rate, blood pressure, and plasma norepinephrine are shown for each subject in table 1 for the pre-study (control) period, for the maximum infusion rate for each drug, and for an intermediate rate for each drug. The maximum nitroprusside infusion rate was 100 μg/min and the intermediate rate was 50 μg/min in all but one subject (subject 2) who received only 25 and 50 μg/min. Four subjects (subjects 1, 2, 5, and 7) received 100 μg/min of phenylephrine with an intermediate rate of 50 μg/min, while three (subjects 3, 4, and 6) received 75 μg/min with an intermediate rate of 50 μg/min.

Table 2 shows the mean values for the control period, the two infusion rates for each drug, and percentage change from control. Nitroprusside caused a significant fall in diastolic (p < 0.01) and mean blood pressure (p < 0.005) at the maximum infusion rate with virtually no effect on systolic pressure. Heart rate

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Control</th>
<th>Intermediate infusion rate</th>
<th>Maximum infusion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroprusside infusion:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>71.6±6.1</td>
<td>82.6±6.0 (+13.5±3.1)</td>
<td>88.4±7.9 (+24.0±7.1)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>98.0±9.8</td>
<td>99.1±11.7 (+0.95±3.8)</td>
<td>96.6±11.1 (-1.7±3.1)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>72.6±9.4</td>
<td>66.7±7.4 (-5.2±5.5)</td>
<td>62.1±10.2 (-14.5±6.4)</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>81.1±9.0</td>
<td>79.1±8.5 (-2.5±2.9)</td>
<td>73.6±10.1 (-13.2±7.0)</td>
</tr>
<tr>
<td>NE, pg/ml</td>
<td>179.0±47.7</td>
<td>267.0±59.6 (+30.2±22.3)</td>
<td>385.6±96.6 (+119.6±46.6)</td>
</tr>
<tr>
<td>Phenylephrine infusion:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>71.6±6.1</td>
<td>61.7±7.7 (-16.4±7.9)</td>
<td>52.1±8.6 (-26.0±7.7)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>98.0±9.8</td>
<td>110.9±11.2 (+11.3±8.3)</td>
<td>124.9±7.3 (+21.6±5.6)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>72.6±9.4</td>
<td>79.7±9.3 (+8.8±8.0)</td>
<td>85.1±7.7 (+18.0±4.7)</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>81.1±9.0</td>
<td>90.0±9.6 (+9.6±7.8)</td>
<td>98.4±6.1 (+17.7±4.7)</td>
</tr>
<tr>
<td>NE, pg/ml</td>
<td>179.0±47.7</td>
<td>123.6±57.1 (-53.3±93.7)</td>
<td>133.6±43.5 (-41.7±27.2)</td>
</tr>
</tbody>
</table>

HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; NE = plasma norepinephrine concentration; numbers in parentheses show percent change from control values.
Figure 1. Blood pressure, heart rate, and plasma norepinephrine during the control period and after 10 minutes of constant infusion of nitroprusside (N) and phenylephrine (P) at two different rates. Values are the means ± SD for the seven subjects (*p < 0.05 vs control; †p < 0.01 vs control).

Figure 2. Change in heart rate (mean ± SD) with change in diastolic blood pressure (mean) during the two infusion rates of nitroprusside and phenylephrine (*p < 0.05 vs control; †p < 0.01 vs control).

Figure 3. Change in plasma norepinephrine (mean ± SD) with the change in diastolic blood pressure (mean) during the two infusion rates of nitroprusside and phenylephrine (*p < 0.05 vs control; †p < 0.01 vs control; N = nitroprusside; P = phenylephrine).
increased with the intermediate \( p < 0.05 \) and maximum \( p < 0.001 \) rates. Plasma norepinephrine increased significantly \( p < 0.001 \) at the highest rate (figs. 1, 2, and 3).

With phenylephrine, all three blood pressure measurements increased significantly during the maximum infusion rate (systolic and diastolic, \( p < 0.005 \); mean, \( p < 0.001 \)), while heart rate fell \( p < 0.001 \) (figs. 1 and 2). Plasma norepinephrine fell during the intermediate infusion rate \( p < 0.025 \) but showed no further reduction with the maximum infusion (figs. 1 and 3).

The magnitude of the change in heart rate, diastolic and mean blood pressure was similar for the two drugs, yet the increase in norepinephrine during nitroprusside hypotension was significantly greater than the decrease with phenylephrine hypertension at the highest doses used \( p = 0.02 \).

For each subject, the change in plasma norepinephrine did not correlate directly with changes in blood pressure or heart rate, but the changes were directionally consistent in all. Figure 4 left expresses the ratio of the mean change in heart rate to a change in diastolic blood pressure, analogous to earlier studies of baroreflex function by Bristow and coworkers\(^7\) in hypertensive subjects. The ratios of changes in plasma norepinephrine to changes in heart rate and to diastolic pressure during the two treatments are compared in figure 4 center and 4 right, illustrating the difference in the sympathetic nervous system response to hypotension and hypertension \( p = 0.02 \).

**Discussion**

We have developed a method of testing baroreceptor-mediated changes in sympathetic vasomotor outflow using plasma norepinephrine responses to nitroprusside hypotension and phenylephrine hypertension in normotensive subjects. Previous investigations of human baroreflexes have largely involved heart rate changes that are mainly under vagal control.\(^1\)-\(^6\) These studies form the basis for the theory that the baroreflexes are "reset" in human hypertension.\(^7\)-\(^11\) It would appear important to obtain some measure of sympathetic vasomotor outflow. Direct recording of peripheral nerve electrical activity in normal and hypertensive subjects has been done during spontaneous fluctuations in blood pressure\(^14\) but this is a relatively cumbersome and invasive method to use in large-scale studies of human baroreceptor function.

Circulating norepinephrine concentration has been proposed as a reflection of peripheral sympathetic ner-

![Image](http://hyper.ahajournals.org/)
vous system activity because that which is free in the circulation is derived almost entirely from the norepinephrine released at the sympathetic nerve terminal. Moment-to-moment changes in activity can be monitored because of the short half-life of norepinephrine in plasma (2 to 3 minutes). Resting or basal levels of plasma norepinephrine have been shown to be relatively constant in individual subjects over several months. That circulating norepinephrine reflects sympathetic activity is also inferred from the observation that levels increase during maneuvers known to stimulate the sympathetic nervous system, such as orthostasis, exercise, hand grip, exposure to cold, and various behavioral stimuli.

Nitroprusside was chosen to produce hypotension and phenylephrine to produce hypertension because of a lack of known direct cardiac or central nervous system effects and relatively short half-lives. A constant infusion, rather than rapid injection as in earlier studies, was used for several reasons. First, dose-response titration allowed comparison of the sympathetic response to circulatory changes of equal magnitude but opposite sign. Second, as has been suggested, steady-state conditions have more meaning with regard to circulatory regulation in hypertension. Third, an added measure of safety was introduced to prevent wide swings in blood pressure; this will be more important when subjects with hypertension are studied.

We have chosen the diastolic blood pressure for comparisons. Much of the literature on the baroreflexes has described changes in relation to the diastolic or mean blood pressure, and we found that the diastolic and mean blood pressures (calculated as the diastolic plus one-third of the pulse pressure) were interchangeable in our data analysis. We also found that we were unable to cause much change in systolic blood pressure with nitroprusside in the doses administered, probably because vasodilation involves activation of sympathetic fibers to both the heart and peripheral vasculature, which tends to maintain the systolic blood pressure.

The chronotropic response to moderate blood pressure changes is largely vagally mediated in the resting state. Leon and coworkers demonstrated this fact in normal volunteers by abolishing the heart rate response to amyl nitrite inhalation, phenylephrine infusion, or the Valsalva maneuver with atropine but were unable to do so with propranolol. Likewise, Greene and Bachand showed no attenuation of the bradycardia to pharmacologically induced blood pressure elevation during sympathetic blockage produced by spinal anesthesia in man. Addition of atropine did block the response.

A variable-pressure neck chamber has been developed to study the blood pressure response to changes in carotid sinus transmural pressure. The response may represent a distinct limb of the baroreflex arc. Using this technique, Mancia and colleagues demonstrated a rise in arterial blood pressure during decreases in carotid sinus stimulation and a lesser fall in blood pressure during an increase in stimulation. These responses are presumably mediated by sympathetic vasomotor fibers since there is no peripheral vascular parasympathetic innervation (however, no autonomic blockade was induced). Our results are compatible with those of this and other studies in that plasma norepinephrine increased to a much greater degree to an induced fall in blood pressure than it decreased during blood pressure elevation. Mancia and coworkers also demonstrated the appropriate changes in heart rate, but of a lesser magnitude than changes in blood pressure; they also compared the heart rate response to that induced by phenylephrine or nitroglycerin and reported larger changes with pharmacologic manipulation. This suggests that extracarotid baroreceptors are more important in the regulation of heart rate and that carotid baroreceptors are more important in blood pressure regulation.

The similar magnitude of response in heart rate to either blood pressure elevation or reduction is shown in figures 2 and 4. This is contrasted with the greater sympathetic nervous system stimulation with hypotension than suppression with blood pressure elevation (figs. 3 and 4 center and right).

Whereas vasodilation activates sympathetic outflow to the heart (tachycardia) and periphery (increase in plasma norepinephrine), in response to pressor stimuli the bradycardic response is greater than the suppression of plasma norepinephrine, supporting the view that the bradycardia is vagally mediated. The relatively small, but consistent, decrease in plasma norepinephrine during pressor stimuli suggests that a withdrawal or suppression of peripheral sympathetic tone is not a potent homeostatic mechanism. Figure 4 summarizes these findings by comparing the changes in heart rate, diastolic blood pressure, and norepinephrine for the two opposing stimuli.

We propose that the method described is safe and simple and may be useful in measuring baroreceptor-mediated changes in sympathetic vasomotor tone. We are planning to apply it to the study of hypertensive patients to further clarify the role of the baroreflexes in the genesis and maintenance of essential hypertension.

References
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Plasma norepinephrine in the evaluation of baroreceptor function in humans.
S H Grossman, D Davis, J C Gunnells and D G Shand

Hypertension. 1982;4:566-571
doi: 10.1161/01.HYP.4.4.566
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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