Baroreflex Sensitivity Modulates Vasodepressor Response to Nitroprusside

ALEXANDER M. M. SHEPHERD, M.D., PH.D., MIN-SHUNG LIN, M.D., JOHN L. MCNAY, M.D., GARY E. MUSGRAVE, PH.D., AND T. KENT KEETON, PH.D.

SUMMARY  Baroreflex activity is a determinant of the homeostatic response to alteration in blood pressure. We examined the factors that determine the magnitude of the vasodepressor response to sequential incremental intravenous infusions of sodium nitroprusside (NP), 0.05 to 6.4 μg/kg/min, in eight male patients with essential hypertension. Each infusion level was of 10 minutes’ duration. Change from control values of mean arterial pressure (ΔMAP), heart rate (ΔHR) and plasma norepinephrine (ΔNE) were obtained at the end of each infusion level. Significant correlations were found between ΔMAP vs log dose NP, ΔHR vs ΔMAP and ΔNE vs ΔMAP for each patient (p < 0.05). However, the slopes of these relationships varied widely between subjects and were significantly correlated with the control blood pressure of each patient. In addition, the sympathetic responsiveness, as measured by ΔNE vs ΔMAP, was inversely correlated with the degree of vasodepressor response seen. Thus, the magnitude of the vasodepressor response was determined by two major factors: 1) the predrug blood pressure, possibly reflecting altered vascular geometry with hypertension; 2) the degree of sympathetic response, which probably acts by mediating the degree of reflex alpha-adrenergic-mediated arteriolar vasoconstriction. (Hypertension 5: 79-85, 1983)

KEY WORDS  * plasma norepinephrine • hypertension • heart rate • humans

The level of baroreceptor activity, acting by way of the sympathetic nervous system, is a major determinant of the homeostatic response to perturbation of blood pressure. Changes in sensitivity of the baroreflex arc occur in hypertension.1-5 sleep,6 and with aging.2,3,5

Baroreflex sensitivity (BRS) is usually expressed in terms of the change in heart rate (HR), or R-R interval, for each unit of change in blood pressure.1,2,5-9 However, there are practical and theoretical limitations to this approach. First, in subjects receiving beta-adrenoceptor antagonists, the relationship between HR response and the level of sympathetic activity may change. Second, HR is controlled by a balance between the level of activity of the vagal and the sympathetic efferent nerves to the heart, and cardiovascular stress causes alteration in both vagal and sympathetic nerve activity.10-12 Accordingly, the use of changes in HR vs changes in blood pressure to measure BRS does not permit a separate examination of these two neural components. Third, alteration in the sensitivity of the cardiac beta-adrenoceptors to norepinephrine (NE) may contribute to variations in the HR versus blood pressure relationship. Finally, only the cardiac effects of alteration in sympathetic nerve activity are measured; therefore, those factors influencing the noradrenergic control of the resistance vessels are not included in this measure of BRS. Since peripheral vascular tone is affected by the sympathetic but not the parasympathetic nervous system, the neural regulation of vascular tone might be better reflected by plasma NE levels.

Plasma NE concentration is considered to be a useful quantitative measure of the level of activity of the sympathetic nervous system, particularly during cardiovascular stress produced by isometric10,11 or dynamic12-16 exercise, tilt,17 change from recumbency to upright posture,18,19 exposure to cold,17,18,20 induction of hypoglycemia,21 the Valsalva maneuver,16 and sodium balance.16,22,23
To circumvent the limitations associated with using change in HR as an index of BRS, we used changes in the plasma concentration of NE to gauge the sympathetic component of the BRS in supine hypertensive patients. In addition, we measured the correlation between the level of sympathetic responsiveness as measured by plasma NE increments and the magnitude of the vasodepressor response seen after administration of sodium nitroprusside (NP). The baroreflex arc was activated by sequential stepwise reductions in blood pressure produced by incremental intravenous infusion rates of NP. This method provides a graded series of measurable stimuli for baroreflex activation. At each level of blood pressure reduction, HR and plasma NE and epinephrine (EP) concentrations were measured and compared with control values.

Methods

Eight patients (aged 45–55 years, weighing 85–116 kg) with essential hypertension were studied in hospital. All patients were receiving long-term therapy for their hypertension but antihypertensive medications except hydrochlorothiazide were stopped at least 6 days prior to the study.

The patients were hospitalized for at least 4 days at bed rest to permit stabilization of blood pressure. Their hospital diet contained 137 mEq of sodium per day. Electrolyte balance was not quantitated; however, no consistent change of body weight was noted. The patients received nothing by mouth except water, beginning the previous midnight. The patients remained recumbent in bed without smoking throughout the study period. At 7 a.m., a pediatric scalp vein needle was placed in a forearm vein for blood sampling. A 5% solution of dextrose and water (D5W) was slowly infused through a second intravenous needle in the other arm which was used for NP infusion. Starting at 9 a.m., each patient was given serial incremental infusions of NP, each of 10 minutes’ duration, by variable speed Harvard infusion pump into the side arm of the D5W infusion run at 0.5 ml/min. Total fluid volume given was less than 50 ml over the 80-minute study. The NP infusion rate was increased over the range of 0.05–6.4 µg/kg/min, or until diastolic pressure was reduced to the level observed during the outpatient series of measurable stimuli for baroreflex activation.

Venous blood was drawn through the scalp vein needle during the last 2 minutes of the control period and during the 9th minute of each infusion period. The scalp vein needle was kept patent with 0.15 M saline containing 10 units heparin/ml. Samples were drawn into 5 ml plastic syringes and immediately transferred to precooled tubes containing EGTA (60 µg/ml) and reduced gluthathione (90 µg/ml). Plasma was separated at 4°C and stored at −70°C until analyzed for NE and EP by a single isotope radioenzymatic assay.

Data Analysis

Blood pressure values are expressed as mean arterial pressure (MAP) calculated as diastolic pressure plus one-third of pulse pressure. Analysis of variance indicated no significant variation in MAP during the 2 hours prior to NP infusion. Control MAP (CMAP) was expressed as the average of the 12 values obtained at 10-minute intervals prior to the infusion of NP. A change in MAP (ΔMAP) was taken to be the difference between CMAP and the average of the last three blood pressure values recorded during each infusion period. Control HR and the change in HR (ΔHR) were obtained at the same time intervals. Plasma NE and EP concentrations were the means of duplicate determinations. Changes in plasma NE (ΔNE) and EP (ΔEP) were the differences between the control values and the values obtained in the ninth minute of each infusion period.

Baroreflex sensitivity was calculated as the ratio of ΔHR to ΔMAP, similar to the steady-state method, which has been shown to be equally reliable with the ramp method.

Statistical analysis was performed by the methods specified in the text. These methods included one-way analysis of variance, least squares linear regression analysis, multiple linear stepwise regression analysis and Z transformation of the correlation coefficient, r. Statistical significance was taken to be p < 0.05.

Catecholamine Assay

This assay technique has previously been described in detail. Briefly, 100 µL of plasma was added to a solution containing buffer, catechol-O-methyltransferase and 3H-S-adenosylmethionine. After the stoichiometric transfer of 3H-methyl groups to the catecholamine by catechol-O-methyltransferase (incubation at 37°C for 60 minutes), the 3H-O-methylated metabolites of NE and EP were separated by a rapid thin layer chromatographic procedure. This isolation procedure prior to counting the 3H-O-methylated metabolites permits a high degree of specificity in the differential assay of NE and EP. The intraassay and interassay coefficients of variation are 6% and 11% respectively, for both NE and EP. The limit of detection is 20 pg/ml for both compounds.

Results

By using infusion rates of NP ranging from 0.05 to 6.4 µg/kg·min, an average of six (range, five to nine) stepwise decrements in MAP were elicited in each patient. The maximal reduction in MAP at the highest rate of infusion averaged 25 mm Hg and ranged from 14 mm Hg in the least hypertensive patient to 40 mm Hg in the most hypertensive patient. Linear regression analysis of data obtained from each patient revealed a significant log dose-response relationship between ΔMAP and the log of the infusion rate of NP (p ≤ 0.005; fig. 1).

The maximal rise in HR during NP-induced vasodepression averaged 26 beats/min (range, 18–38 beats/min). Within each subject, a significant positive corre-
CATECHOLAMINE RESPONSE TO NITROPRUSSIDE/Shepherd et al. 81

**Figure 1.** Individual plots of the best-fit calculated regression lines relating the logarithm of the infusion rate of sodium nitroprusside (NP) to the vasodepressor response seen.

**Figure 2.** Individual plots of the best-fit calculated regression lines relating the vasodepressor response seen to (a) the rise in heart rate (HR) and (b) the rise in plasma norepinephrine (NE) concentration.

**Figure 3.** Individual plots of the best-fit calculated regression lines relating the rise in plasma norepinephrine (NE) concentration to the rise in heart rate (HR).

lution \( p \leq 0.01 \) existed between the decrease in MAP and the resulting increase in HR (fig. 2a).

The mean plasma NE concentration prior to the administration of NP was 199 pg/ml (range, 162–435 pg/ml). No relationship was found between the severity of the hypertension and the baseline levels of either NE \( r = 0.5503 \) or EP \( r = -0.1550 \) in the plasma. The stepwise decrements in MAP caused by increasing doses of NP were accompanied by stepwise increments in plasma NE concentration in all subjects (fig. 2b). Moreover, a correlation existed between the rise in plasma NE concentration and the corresponding increase in HR at each infusion rate of NP in each patient (fig. 3). In only one subject was a significant correlation found between rise in EP and ΔMAP. The maximum rise in plasma EP was small \( 33 \pm 6 \) pg/ml, mean \( \pm \) SEM compared with that of NE \( 275 \pm 84 \) pg/ml.

Although correlations were noted between ΔMAP/log dose NP, ΔHR/ΔMAP, ΔNE/ΔMAP and ΔHR/ΔNE within the data of each patient, a considerable amount of interindividual variation was found in the slopes of these relationships. For example, in some patients a small fall in MAP was associated with a
large increase in plasma NE concentration, whereas in other patients, a large decrease in MAP was accompanied by only a small rise in plasma NE levels. The slopes of the best-fit regression lines for each subject are shown in table 1.

To determine whether CMAP was related to the slopes of these relationships, the slopes of the best-fit regression lines of each of these relationships were correlated against CMAP for each patient. This method of analysis revealed a positive correlation between CMAP and the slope of ΔMAP/log₁₀ NP dose (fig. 4 a):

\[ \Delta \text{MAP}/\log_{10} \text{NP dose} = -56.0 + 0.67 \text{CMAP}, \]

where \( r = 0.8913, p = 0.0014 \).

A negative correlation was noted between CMAP and the slopes of both ΔHR/ΔMAP (fig. 4 b) and ΔNE/ΔMAP (fig. 4 c):

\[ \Delta \text{HR}/\Delta \text{MAP} = 4.1 - 0.03 \text{CMAP}, \]

where \( r = 0.8052, p = 0.0128 \).

\[ \Delta \text{NE}/\Delta \text{MAP} = 51.1 - 0.32 \text{CMAP}, \]

where \( r = 0.6347, p = 0.0935 \).

Last, a positive correlation existed between CMAP and the slope of the ΔHR/ΔNE relationships (fig. 4 d):

\[ \Delta \text{HR}/\Delta \text{NE} = -0.033 + 0.001 \text{CMAP}, \]

where \( r = 0.6925, p = 0.0565 \).

The last two relationships failed to reach statistical significance.

To determine whether the slopes of the individual dose response relationships were influenced by BRS or by the reactivity of the sympathetic nervous system, the slopes of the dose response curves were regressed against ΔHR/ΔMAP (fig. 5 a) and against ΔNE/ΔMAP/log₁₀ NP dose (fig. 5 b).

### Table 1. CMAP and the Slopes of the Relationships Between the Measured Parameters

<table>
<thead>
<tr>
<th>Subject</th>
<th>CMAP (mm Hg)</th>
<th>ΔMAP/log₁₀ NP dose</th>
<th>ΔHR/ΔMAP</th>
<th>ΔNE/ΔMAP</th>
<th>ΔHR/ΔNE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98</td>
<td>10.0</td>
<td>1.85</td>
<td>18.6</td>
<td>0.087</td>
</tr>
<tr>
<td>2</td>
<td>107</td>
<td>17.8</td>
<td>1.36</td>
<td>14.0</td>
<td>0.070</td>
</tr>
<tr>
<td>3</td>
<td>108</td>
<td>15.3</td>
<td>1.57</td>
<td>30.5</td>
<td>0.055</td>
</tr>
<tr>
<td>4</td>
<td>109</td>
<td>15.5</td>
<td>1.40</td>
<td>12.1</td>
<td>0.107</td>
</tr>
<tr>
<td>5</td>
<td>113</td>
<td>13.2</td>
<td>1.30</td>
<td>9.4</td>
<td>0.080</td>
</tr>
<tr>
<td>6</td>
<td>123</td>
<td>37.4</td>
<td>0.33</td>
<td>4.3</td>
<td>0.081</td>
</tr>
<tr>
<td>7</td>
<td>131</td>
<td>23.5</td>
<td>1.20</td>
<td>13.3</td>
<td>0.087</td>
</tr>
<tr>
<td>8</td>
<td>152</td>
<td>46.4</td>
<td>0.41</td>
<td>2.37</td>
<td>0.140</td>
</tr>
</tbody>
</table>

CMAP = control mean arterial pressure; ΔMAP = change in mean arterial pressure from control; NP = nitroprusside; ΔHR = change in heart rate; ΔNE = change in plasma norepinephrine.

### Figure 4

Individual data, with calculated best-fit regression lines relating the baseline arterial blood pressure (CMAP) to the slopes of the relationships: (a) vasodepressor response vs logarithm of sodium nitroprusside (NP) infusion rate; (b) rise in heart rate (HR) vs vasodepressor response; (c) rise in plasma norepinephrine (NE) concentration vs vasodepressor response; (d) rise in HR vs rise in plasma NE concentration.
\[ \text{AMAP/log}_{10} \text{NP dose} = 49.2 - 22.7 \text{AHR/AMAP}, \]
\[ \text{where } r = 0.9468, p < 0.0001; \]
\[ \text{AMAP/log}_{10} \text{NP dose} = 35.5 - 1.00 \text{ANE/AMAP}, \]
\[ \text{where } r = 0.6870, p = 0.0590. \]

Since the parameters CMAP, AHR/AMAP and ANE/AMAP were likely to co-vary, multiple linear stepwise regression analysis was undertaken with AMAP/log\(_{10}\)NP dose as the dependent variable and CMAP, AHR/AMAP, ANE/AMAP as independent variables. The following regression equation was obtained:

\[ \text{AMAP/log}_{10} \text{NP dose} = 8.57 + 0.27 \text{CMAP} - 15.64 \text{AHR/AMAP}, \]
\[ \text{where } r = 0.9714, p < 0.0001. \]

This indicates that both CMAP and AHR/AMAP were independent determinants of the slope of the dose response curve.

To determine if CMAP or BRS (expressed as AHR/AMAP) was related to the extrapolated threshold values of the regression lines, AMAP vs log dose NP, AHR vs AMAP, and ANE vs AMAP, the threshold values were correlated against CMAP for each patient. No significant correlations could be found between CMAP and the relationships; AMAP vs log dose NP \((p = 0.40)\); AHR vs AMAP \((p = 0.60)\); ANE vs AMAP \((p = 0.50)\).

**Discussion**

In the present study we have shown a positive correlation between slope of the NP vs blood pressure dose response curves and the predural blood pressure. Similar relationships have been found for other vasodilators including diazoxide\(^25\) and minoxidil\(^26\) and for diuretics.\(^27\)\(^-\)\(^29\) We could not detect any interrelationship between the threshold dose of nitroprusside required and the baseline blood pressure. This supports the work of Sivertsson,\(^30\) who found no evidence of supersensitivity in the hand resistance vessels of hypertensive patients. Increased vascular sensitivity might be expected to be reflected in a shift of the threshold dose of vasodilator required to cause fall in pressure.

Despite the potential limitations, already discussed, to using change in HR as a measure of baroreflex sensitivity, we were able to demonstrate good relationships between the slope of the dose-response relationship and both AHR/AMAP and baseline blood pressure. A relationship between baroreflex sensitivity and baseline blood pressure has been previously demonstrated.\(^31\) However, the relationship between baroreflex sensitivity and vasodepressor responsiveness has not been previously demonstrated. It indicates an association between this measure of baroreflex sensitivity and the degree of homeostatic responsiveness to reduction in blood pressure.

The sympathetic nervous system is known to play an important role in the control of the circulation. However, it is difficult to assess the basal level of activity of the sympathetic nervous system in clinical studies. Although the plasma concentration of NE is generally regarded as a useful measurement of the level of stimulation of the sympathetic nervous system, the resting levels of plasma NE do not necessarily reflect sympathetic nervous system activity. This is probably in part due to the levels of NE in the blood, which are not determined solely by the rate of spillover from peripheral sympathetic nerves into the circulation. Additional factors influencing the plasma NE levels include variation in distribution and rate of metabolism, which
is seen in normal subjects, hypertensive patients, autonomic insufficiency, and following drug interventions. It may be for this reason that there is dispute as to the interrelationship between baseline plasma NE levels and the presence and extent of hypertension. However, plasma NE levels are considered to be representative of sympathetic nerve activity. The failure of plasma EP to rise significantly indicates that arterial baroreceptors, potentiates the vasodepressor effect of veratrum alkaloid, cryptenamine, which acts on baroreceptors, potentiates the vasodepressor effect of NE. Greenway and Innes found that carotid reflex mechanisms reduced the vasodepressor response of anesthetized vagotomized cats to intravenous NP by approximately two-thirds. The principal mechanism of this physiological antagonism was defined as an increase in peripheral resistance. A reflex increase in iliac resistance during NP infusion in conscious dogs has been clearly demonstrated by Pagani et al. Accordingly, activation of vascular alpha-adrenoceptors may be a major mechanism by which autonomic reflexes modulate the vasodepressor response to vasodilator drugs. By contrast, the hemodynamic effects of altered autonomic control of heart rate and contractility may be relatively minor, since combined pretreatment with atropine and propranolol does not potentiate the vasodepressor response of supine hypertensive patients to intravenous bolus doses of diazoxide.

In conclusion, we have demonstrated associations between the degree of vasodepressor response seen with graded infusions of NP and both the baroreflex sensitivity and baseline blood pressure. The latter association possibly reflects structural vascular changes related to the degree of hypertension. In addition, the data are consistent with the conclusion that adrenergically mediated vasoconstriction antagonizes the hypertensive response to nitroprusside in supine hypertensive patients.

References

11. Eckberg DL: Carotid baroreceptor stimulus-sinus node re-


Baroreflex sensitivity modulates vasodepressor response to nitroprusside.
A M Shepherd, M S Lin, J L McNay, G E Musgrave and T K Keeton

Hypertension. 1983;5:79-85
doi: 10.1161/01.HYP.5.1.79

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/1/79