Dissociation Between Neural and Vascular Responses to Sympathetic Stimulation

Contribution of Local Adrenergic Receptor Function

Giris Jacob, Fernando Costa, John Shannon, David Robertson, Italo Biaggioni

Abstract—Sympathetic activation produced by various stimuli, eg, mental stress or handgrip, evokes regional vascular responses that are often nonhomogeneous. This phenomenon is believed to be the consequence of the recruitment of differential central neural pathways or of a sympathetically mediated vasodilation. The purpose of this study was to determine whether a similar heterogeneous response occurs with cold pressor stimulation and to test the hypothesis that local differences in adrenergic receptor function could be in part responsible for this diversity. In 8 healthy subjects, local norepinephrine spillover and blood flow were measured in arms and legs at baseline and during sympathetic stimulation induced by baroreflex mechanisms (nitroprusside infusion) or cold pressor stimulation. At baseline, legs had higher vascular resistance ($27 \pm 5$ versus $17 \pm 2$ U, $P=0.05$) despite lower norepinephrine spillover ($0.28 \pm 0.04$ versus $0.4 \pm 0.05$ mg $\cdot$ min$^{-1}$ $\cdot$ dL$^{-1}$, $P=0.03$). Norepinephrine spillover increased similarly in both arms and legs during nitroprusside infusion and cold pressor stimulation. On the other hand, during cold stimulation, vascular resistance increased in arms but not in legs ($20 \pm 9\%$ versus $-7 \pm 4\%$, $P=0.03$). Increasing doses of isoproterenol and phenylephrine were infused intra-arterially in arms and legs to estimate $\beta$-mediated vasodilation and $\alpha$-induced vasoconstriction, respectively. $\beta$-Mediated vasodilation was significantly lower in legs compared with arms. Thus, we report a dissociation between norepinephrine spillover and vascular responses to cold stress in lower limbs characterized by a paradoxical decrease in local resistance despite increases in sympathetic activity. The differences observed in adrenergic receptor responses cannot explain this phenomenon. (Hypertension. 2000;35:76-81.)

Key Words: autonomic nervous system $\bullet$ norepinephrine $\bullet$ receptors, adrenergic $\bullet$ vasodilation

It is generally accepted that the sympathetic nervous system exerts a differentiated control on the various body organs. This postulate is supported primarily from animal studies using direct recordings of sympathetic nerve traffic showing differential activation of various vascular beds. Similarly, a heterogeneous pattern of sympathetic activation has been shown in humans by use of regional norepinephrine spillover or changes in vascular resistance, eg, in upper and lower limbs, during various stimuli. It is possible that this nonhomogeneous sympathetic activation is due to discrete neural pathways evoked by the different stimuli. This would increase sympathetic nerve activity with a corresponding elevation in vascular resistance in some vascular beds but not in others.

A second layer of complexity is introduced by the apparent dissociation observed in some instances between sympathetic activation, regional norepinephrine spillover, and their corresponding change in vascular tone. For instance, muscle sympathetic nerve activity was found to be similar in both arms and legs at rest, whereas a lower norepinephrine spillover has been found in legs compared with arms. Sustained handgrip causes vasoconstriction in legs but vasodilation in the contralateral arm, whereas sympathetic nerve activity increases similarly in both radial and peroneal nerve during this stimulus. Differential vascular responses to other stimuli, such as mental stress test, local ischemia, and coughing, have been reported when arms and legs are compared, but no data are available on simultaneous measurements of sympathetic activity.

It has been suggested that neurally mediated vasodilation may account for the dissociation between increased sympathetic activity and the paradoxical vasodilation. Another potential explanation of these findings may lie in the receptors that mediate vascular responses to sympathetic activation and, in particular, in the balance between $\alpha$-mediated vasoconstriction and $\beta$-mediated vasodilation. There are several
studies exploring local vascular adrenergic receptor function in the forearm, but we are not aware of similar studies in lower limbs.

The present study was designed to examine the differential effect of central sympathetic activation on arms and legs and the vascular consequences of this activation. We used cold stimulation (immersion of a hand in ice water) as a sympathetic stimulus and measured the degree of sympathetic activation (regional norepinephrine spillover) and vascular responses (vascular resistance measurements) on arms and legs. We used the hypotensive effects of nitroprusside as a control stimulus to induce baroreflex-mediated sympathetic activation. Finally, we tested the hypothesis that the diversities in local vascular responses between the extremities could be in part due to dissimilarities in local adrenergic receptor responses. For this purpose, we measured the local vascular responses to intra-arterial infusions of β- and α-adrenergic receptor agonists in arms and legs.

**Methods**

**Subjects**
The study group consisted of 8 healthy subjects (7 females and 1 male) aged 24 to 50 years (30 ± 2 years, median 34 years). Their body mass index was 23.6 ± 0.7 kg/m², and their weight and height were 64 ± 2.5 kg and 1.68 ± 0.025 m, respectively. No subject had a history of alcohol abuse, drug abuse, or smoking. All procedures were approved by the institutional review board, and subjects gave informed consent before the study, in accordance with institutional guidelines.

Subjects were studied after at least 3 days on a diet containing 150 mEq Na\(^+\) and 70 mEq K\(^+\) per day that was free of caffeine and low in mononitramines. This diet resulted in urinary sodium of 121 ± 4 mEq/24 hours and urinary potassium of 58 ± 3 mEq/24 hours. All subjects were admitted to the General Clinical Research Center at Vanderbilt University and studied while they were supine after an overnight fast.

**Protocols**

**Local and Systemic Norepinephrine Spillover and Clearance**

A brachial artery, the ipsilateral femoral vein, and 2 large antecubital veins were catheterized. The arterial line was connected through a 3-way valve to a pressure transducer (DT-4812, Omheda). One port was dedicated to blood sampling and the second was dedicated to flushing with heparinized saline. Access to one antecubital vein was obtained by direct puncture. This limitation is inherent to this technique and is discussed in detail elsewhere. After instrumentation, the subject rested quietly for at least 30 minutes. [3H]NE was prepared and infused as described previously. Blood samples for norepinephrine spillover were taken simultaneously from the brachial artery, the femoral vein, and an antecubital vein. Forearm and calf blood flow was measured by use of venous occlusion plethysmography for 4 seconds at 8-second intervals as described previously. Vascular resistance was calculated as mean arterial blood pressure divided by blood flow and expressed in units of mm Hg · mL \(^{-1} \) · DL tissue \(^{-1} \) · min \(^{-1} \).

Norepinephrine spillover was assessed during nitroprusside infusion and during cold stimulation. Nitroprusside was infused intravenously at increasing doses for 5 minutes each, starting at 0.1 μg · kg \(^{-1} \) · min \(^{-1} \), until a decrease in systolic blood pressure of ~20 mm Hg was achieved. The cold stimulation was induced by submerging the hand contralateral to the flow measurement in iced water (cold pressor test). Measurements were performed during the last minute of nitroprusside infusion and during the 60 to 90 seconds of the cold stimulation. At least 20 minutes elapsed between each stimulus to allow parameters to return to baseline. Separate baseline values of norepinephrine spillover were determined before each intervention.

**Local Adrenergic Receptor Sensitivity**

After a 60-minute rest period, isoproterenol was infused sequentially into the brachial and femoral arteries at increasing doses (from 0 to 300 mg/min). Each dose of isoproterenol was infused for 5 minutes, and blood flow was recorded during the last minute. After a 30-minute rest period, we measured the vasoconstrictive effect of phenylephrine. For this purpose, we first induced sustained vasodilation with isoproterenol at individualized doses that induced ~50% of maximal vasodilation (20 to 40 mg/min for arms and 40 to 60 mg/min for legs). After 15 minutes of this fixed dose of isoproterenol infusion, phenylephrine was administered in increasing doses (0.2 to 12 μg/min). Dose-response curves were constructed, and the maximal effect (E\(_{\text{max}}\)) and the dose of agonist producing half-maximal effect (E\(_{\text{50}}\)) were extrapolated from nonlinear regression of the individual curves. No attempt was made to normalize the dose to the volume of the limb. It would be possible to do this in the forearm, because drugs are infused directly into the brachial artery irrigating the forearm. This, however, cannot be applied to the leg, because flow is measured in the calf, but the infusion is made in the femoral artery. Correction by volume would require drug infusions into the popliteal artery. This limitation is inherent to this technique and is discussed in detail elsewhere.

**Statistical Analysis**

Results are expressed as mean ± SEM. Single comparisons within and between groups were made by use of paired and unpaired 2-tailed t tests, respectively. One-way ANOVA for repeated measurement was used to assess dose-related effects. Nonlinear regression analysis was performed for each dose-response curve. Data were analyzed by use of GraphPad Prism (GraphPad Software Inc). A value of P < 0.05 was considered statistically significant.

**Results**

Cardiovascular and biochemical parameters at baseline and during stimulation are shown in Table 1. There were no significant differences in plasma norepinephrine levels between the sampling site at baseline, but arterial samples tended to be lower (Figure 1a). During nitroprusside infusion, plasma norepinephrine increased significantly and similarly at all sampling sites (by 45 ± 6%, 63 ± 15%, and 65 ± 14% for arm, leg, and artery, respectively; P < 0.001 for all sites; Table 1 and Figure 2a). During cold stimulation, the increment in plasma norepinephrine levels was more pronounced in arms than in the other 2 sampling sites (47 ± 15%, 18 ± 6%, and 16 ± 5%, with P < 0.001, 0.01, and 0.03 for arm, leg, and artery, respectively; Table 2 and Figure 3a). There were no significant differences in mean local blood flow or extraction fraction between arms and legs at baseline (Table 2). Nitroprusside infusion and cold pressor stimuli caused significant changes in systolic and diastolic blood pressure, but extraction fraction and local blood flow did not change significantly, except for an increment in leg blood flow after cold pressor stimulus (paired t test, P = 0.02; Table 2).

Systemic norepinephrine spillover increased significantly during nitroprusside infusion (by 80%) and during cold pressor stimuli (by 45%, Table 1). Systemic clearance did not change after nitroprusside infusion but tended to increase after cold stimulation (P = 0.07).
Local Norepinephrine Spillover and Clearance

Regional norepinephrine spillover was significantly higher at baseline in arms (0.4 \pm 0.05 \text{ ng/min/L} \text{ dL}^{-1}) compared with legs (0.28 \pm 0.04 \text{ ng/min/L} \text{ dL}^{-1}, P<0.03). Similar findings have been reported previously. The mean baseline norepinephrine clearances were similar in both arms and legs (Table 2).

Norepinephrine spillover increased similarly in both arms and legs during nitroprusside infusion (by 0.24 \pm 0.08 and 0.24 \pm 0.07 \text{ mg} \cdot \text{mL}^{-1} \cdot \text{dL}^{-1}, respectively; Figure 2b). Norepinephrine spillover increased less during cold pressure stimuli, but the increase was similar in both extremities (by 0.15 \pm 0.05 and 0.13 \pm 0.05 \text{ mg} \cdot \text{mL}^{-1} \cdot \text{dL}^{-1} in arms and legs, respectively; Figure 3b). Local norepinephrine clearances are shown in Table 2.

Local vascular resistance was lower in arms compared with legs at baseline (17 \pm 2 versus 27 \pm 5 U, P=0.06; Figure 1c). There was a significant correlation between regional norepinephrine spillover and vascular resistance in arms (r=0.75, P<0.02) and legs (r=0.75, P<0.04). Local vascular resistance decreased similarly in both arms and legs during nitroprusside infusion (to 13 \pm 2 and 20 \pm 30 U, paired t test 0.02 and 0.07 for arms and legs, respectively; Figure 2c). In contrast, during cold pressor stimuli, arm vascular resistance increased, whereas leg vascular resistance decreased (4 \pm 2

### Table 1. Hemodynamic and Systemic Catecholamine Responses to Intravenous Nitroprusside Infusion and Cold Stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>NTP</th>
<th>P</th>
<th>Baseline</th>
<th>CPT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>108 \pm 2</td>
<td>85 \pm 2</td>
<td>0.03</td>
<td>109 \pm 2</td>
<td>125 \pm 5</td>
<td>0.005</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>49 \pm 2</td>
<td>39 \pm 2</td>
<td>0.05</td>
<td>50 \pm 2</td>
<td>60 \pm 4</td>
<td>0.05</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>67 \pm 4</td>
<td>81 \pm 4</td>
<td>0.005</td>
<td>66 \pm 3</td>
<td>70 \pm 5</td>
<td>NS</td>
</tr>
<tr>
<td>Arm venous NE, pg/mL</td>
<td>242 \pm 13</td>
<td>340 \pm 22</td>
<td>0.001</td>
<td>242 \pm 13</td>
<td>345 \pm 37</td>
<td>0.025</td>
</tr>
<tr>
<td>Leg venous NE, pg/mL</td>
<td>245 \pm 24</td>
<td>370 \pm 17</td>
<td>0.003</td>
<td>215 \pm 19</td>
<td>250 \pm 16</td>
<td>0.035</td>
</tr>
<tr>
<td>Arterial NE, pg/mL</td>
<td>205 \pm 15</td>
<td>333 \pm 30</td>
<td>0.003</td>
<td>215 \pm 13</td>
<td>248 \pm 12</td>
<td>0.02</td>
</tr>
<tr>
<td>Systemic spillover, ng/min</td>
<td>514 \pm 60</td>
<td>745 \pm 75</td>
<td>0.04</td>
<td>505 \pm 70</td>
<td>670 \pm 70</td>
<td>0.035</td>
</tr>
<tr>
<td>Systemic clearance, L/min</td>
<td>2.28 \pm 0.2</td>
<td>2.3 \pm 0.2</td>
<td>NS</td>
<td>2.3 \pm 0.25</td>
<td>2.65 \pm 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean \pm SEM. NTP indicates sodium nitroprusside infusion (0.95 \pm 0.09 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}); CPT, cold pressor test; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NE, norepinephrine; and NS, nonsignificant. P values are from paired t tests from the previous baseline period.

Figure 1. Baseline values of plasma norepinephrine (NE) (a) in the antecubital vein (arms), femoral vein (legs), and radial artery (artery) and baseline values of regional NE spillover (b) and local vascular resistance (c) in arms and legs. P values indicate differences between arms and legs by 2-tailed unpaired t test.

Figure 2. Increment of plasma NE (a), regional NE spillover (b), and local vascular resistance (c) evoked by the decrease in blood pressure induced by an intravenous infusion of sodium nitroprusside.
versus \(-2\pm1\) U, \(P=0.02\); see Figure 3c). In legs, the correlation between norepinephrine spillover and vascular resistance was maintained \((r=0.8, P=0.02)\) during nitroprusside infusion but not during cold stimulation \((r=0.6, P=NS)\). There was no correlation between the norepinephrine spillover and the local vascular resistance during both stimuli.

**Local Adrenergic Receptor Sensitivity**

Isoproterenol infusion resulted in an increase in forearm blood flow \((3.9\pm0.4 \, \text{range} 2.3 \text{ to } 6.2 \, \text{mL/dL to } 20\pm2 \, \text{range } 9 \text{ to } 27 \, \text{mL/dL})\) and leg blood flow \((4.5\pm9 \, \text{range } 1.9 \text{ to } 7 \, \text{mL/dL to } 10\pm2 \, \text{range } 2.7 \text{ to } 13 \, \text{mL/dL})\) in a dose-dependent fashion, as illustrated in Figure 4a. The maximal dilatatory effect of \(\beta_2\)-adrenergic receptors \((E_{\text{max}})\) was greater in arms than in legs \((435\% \text{ versus } 160\%, \text{ Figure 4a})\).

Stable vasodilation was induced with a constant dose of isoproterenol. The flow obtained after 10 to 15 minutes of infusion was 10\pm1 mL/dL in arms and 6\pm0.9 mL/dL in legs. Phenylephrine caused a vasoconstriction in a dose-response fashion in both arms and legs, as shown in Figure 4b. The \(E_{\text{D}50}\) for phenylephrine was 3-fold greater in legs than in arms. It should be noted, however, that these differences can be explained by the greater tissue volume of the legs, which is \(\approx3\) times greater than that of the arms.3

**Discussion**

The main observations of the present study are the following: First, regional norepinephrine spillover at rest was higher in arms than in legs, whereas vascular resistance was higher in legs. Second, baroreflex-mediated sympathetic stimulation with nitroprusside produced similar increases in local norepinephrine spillover in arms and leg. Third, cold pressor stimulation evoked a similar increase in local norepinephrine spillover in arms and legs, but vascular resistance appropriately increased in arms but decreased in legs instead of increasing. Finally, the effectiveness of \(\beta_2\)-adrenergic receptors to induce vasodilation was significantly lower in legs than in arms.
It is widely accepted that sympathetic activation to various stimuli is not uniformly distributed throughout the body and that some vascular beds may be spared of sympathetically mediated vasoconstriction. The mechanisms underlying this heterogeneous response are not completely understood and may occur at different levels of the organization of the sympathetic nervous system. Animal data indicate specific distribution of efferent nerve traffic to various organs depending on the afferent stimuli, suggesting the recruitment of discrete neural pathways. 

In humans, direct measurement of sympathetic nerve traffic is limited by its accessibility and, therefore, is mostly performed in the peroneal nerve in legs and the radial nerve of arms. It has been shown that mental stress evokes a significant increase in muscle sympathetic activity in the peroneal nerve but not in the radial nerve. 

On the other hand, lower body negative pressure elicited a similar response in both arms and legs. We are not aware of studies in which muscle sympathetic nerve activity has been measured in arms and legs during cold stimulation.

Because of limitations in nerve recording accessibility, most of the knowledge about differential sympathetic activation in humans is based either on regional norepinephrine spillover or on the functional consequences of sympathetic activation, namely, changes in regional vascular resistance. For example, mental stress appears to preferentially increase cardiac norepinephrine spillover, a phenomenon with obvious implications for stress-mediated cardiac abnormalities, including sudden death. On the other hand, aerobic exercise training reduces renal norepinephrine spillover but does not change cardiac spillover. To our knowledge, no data are available about differences in local norepinephrine spillover between arms and legs during any stress stimulus.

Rusch et al have studied extensively the effect of various stimuli on blood flow to arms and legs in humans. Mental stress and static handgrip elicited an increase in heart rate and blood pressure, and this was associated with an increase in forearm but not calf blood flow. These differences in vascular responses between arms and legs were postulated to be due to regional sympathetic cholinergic activation.

In the present study, we have used the combined approach of measuring regional norepinephrine spillover and changes in vascular tone. This approach generated unexpected findings. We found a dissociation between sympathetic activation and its functional correlate. Specifically, we found that cold pressor stimulus produced sympathetic activation in both arms and legs, as determined by regional norepinephrine spillover. This finding agrees with other studies showing an increase in leg muscle sympathetic nerve activity during cold pressor stimulus. In contrast, vascular resistance increased only in arms but not in legs. We are not aware of previous examples of an increase in norepinephrine spillover not associated with an increase in vascular resistance.

Sustained isometric handgrip increases sympathetic nerve traffic in both arms and legs even though it is accepted that this stimulus produces vasoconstriction in legs but vasodilation in the contralateral arm. 

Therefore, there is a precedent for a dissociation between sympathetic nerve traffic and vascular responses as seen in the present study. The mechanism that explains this dissociation is not known, but the possibility of sympathetically mediated vasodilatation involving a nitric oxide mechanism has been proposed.

In the present study, we investigated the possibility of a differential end-organ responsiveness to adrenergic stimulation. We found previously unreported differences in local adrenergic receptor sensitivity between arms and legs. The relatively lower sensitivity of α1-adrenergic receptor-mediated vasoconstriction in legs can be explained by differences in tissue volume; therefore, the relevance of this finding is unclear. In this regard, Streeten found no difference in venous responsiveness to norepinephrine between hand and feet veins in normal subjects. On the other hand, we found a decreased efficacy of β2-adrenergic receptor–mediated vasodilation in legs. Postsynaptic vascular responses, therefore, cannot explain the neural-vascular dissociation found in legs. The significance of this finding was not explored in the present study, but we speculate that the decreased sensitivity to β2-mediated vasodilation in legs contributes to the maintenance of vascular tone during the sympathetic activation associated with upright posture. It is also interesting to note that, at rest, norepinephrine spillover was lower in the leg than in the arm, even though vascular resistance was higher. Taken together, our data would suggest lesser sympathetic control of the arterial circulation of legs compared with arms in humans. Abnormalities in this putative defense mechanism may be of potential relevance to disorders characterized by orthostatic intolerance.

In summary, we report a dissociation between changes in local norepinephrine spillover and vascular tone in legs in response to sympathetic activation evoked by cold stimulation. This dissociation was characterized by a paradoxical decrease in vascular resistance in the leg during cold stimulation, despite an increase in sympathetic activity. Legs were also less responsive to β-mediated vasodilation. Therefore, the differences observed in adrenergic receptor responses cannot explain the neurovascular dissociation produced by cold stress in legs.

References


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