Direct Neurohumoral Evidence for Isolated Sympathetic Nervous System Activation to Skeletal Muscle in Response to Cardiopulmonary Baroreceptor Unloading

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It has been postulated that cardiopulmonary baroreceptor unloading in humans results in nonuniform activation of the sympathetic nervous system. We reasoned that simultaneous measurements of arterial and venous norepinephrine (NE) spillover and clearance (using NE kinetics), muscle sympathetic neural activity (using microneurography), forearm blood flow (using plethysmography), and skin blood flow (using laser Doppler velocimetry) during lower body negative pressure at −15 mm Hg would isolate the location and extent of cardiopulmonary baroreceptor–mediated sympathetic nervous system activation. We exposed normal subjects (n=8) to lower body negative pressure for 30 minutes, with measurements obtained at baseline, 5–10 minutes (EARLY), and 25–30 minutes (LATE). We found that arterial NE spillover, reflecting sympathetic nervous system activation, did not increase significantly, whereas arterial NE clearance decreased significantly. In contrast, forearm venous NE spillover, reflecting skin and muscle sympathetic nervous system activation, increased by 17% and muscle sympathetic neural activity by 35% EARLY, whereas venous clearance did not change significantly. Although laser Doppler skin blood flow did not change, plethysmographic forearm blood flow (combined muscle and skin blood flow) decreased by 28%. All changes were sustained throughout 30 minutes of lower body negative pressure. Our data suggest that sympathetic vasoconstriction to muscle is greater than it is in skin response to cardiopulmonary baroreceptor unloading. Moreover, our data suggest that reduced NE clearance in the arterial circulation is the primary mechanism by which arterial NE concentrations rise. Conversely, NE spillover appears to be the primary mechanism responsible for increasing venous NE concentrations measured from the forearm during cardiopulmonary baroreceptor unloading. (Circulation Research 1990;66:1720–1728)

Isolated unloading of cardiopulmonary baroreceptor tone in humans is purported to result in a nonuniform activation of the sympathetic nervous system. Lower body negative pressure (LBNP) is a relatively simple and effective technique used to assess baroreflex function—in particular, the function of low-pressure baroreceptors.1–3 Johnson et al1 have demonstrated that with less than 20 mm Hg negative pressure, the heart rate, aortic pulse pressure, and mean arterial pressure remained unchanged, but right atrial pressure decreased. This was associated with reductions in plethysmographically determined forearm blood flow and splanchnic blood flow. These findings indicate that LBNP of less than 20 mm Hg negative pressure probably unloads only low-pressure baroreceptors.

In addition, several authors, using microneurographic techniques, have shown that low levels of LBNP increase nerve traffic to limb skeletal muscle.3–5 This increased efferent activity is postulated to be the neural counterpart of forearm vasoconstriction demonstrated plethysmographically.3 Although microneurographic techniques and plethysmographic measures of forearm blood flow permit an assessment of postganglionic nerve traffic and the vascular alterations that occur in response to

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LBPN, they do not directly examine activity at the neuroeffector junction. Norepinephrine (NE) spillover has been shown to be a better index of sympathetic nervous system activation and more accurately assesses neuroeffector junction activity than measures of plasma NE alone. Recently, a sensitive tritiated ([3H]) NE radiotracer technique has been used to quanititate spillover of NE to plasma during orthostatic stress, which is known to unload both low- and high-pressure baroreceptors. To date, studies examining the effect of low-pressure baroreceptor unloading on NE spillover and clearance have not been done.

The purpose of this study was to measure systemic arterial and forearm venous NE spillover and clearance and to measure muscle sympathetic nerve traffic simultaneously. In addition, we also measured plethysmographic forearm blood flow (skin and muscle) and laser Doppler velocimetric blood flow to assess the vasoconstriction responses in each vascular bed during LBNP. In this way, we could examine in detail multiple indexes of sympathetic nervous system efferent limb function in response to isolated cardiopulmonary baroreceptor unloading. We postulated that the use of multiple modalities would yield important information regarding the extent and localization of sympathetic nerve efferent limb activity that results from unloading low-pressure baroreceptors.

Subjects and Methods

Subjects

The study group consisted of eight normal subjects with a mean age of 25±1.4 years (±SEM) (range, 22–33 years). The protocol was reviewed and approved by the clinical investigation committee of the Milton S. Hershey Medical Center. Informed consent was obtained from each individual.

No subject enrolled in the study had a history of heart disease, hypertension, or thyroid disease, and no subject was taking medications or substances that might have altered sympathetic nervous system activity.

A separate group of five subjects (two normal subjects and three heart-failure patients) was studied using continuous-infusion [3H]NE in a supine, resting position for 80 minutes. Blood samples were obtained at 20-minute intervals from initiation of constant infusion to determine whether progressive directional changes in [3H]NE counts occurred over this period of time.

Experimental Protocol

Subjects were comfortably positioned supine in an LBNP chamber. Polyethylene wrap was used to obtain an airtight seal at the level of the iliac crests. The studies were performed in a quiet, partially darkened, temperature-controlled room. The subjects were instrumented with bilateral, 5.71-cm, 16-gauge antecubital venous cannulae for infusion of [3H]NE in the left arm and contralaterally in the antecubital fossa of the right arm for venous blood sampling. A left radial arterial cannula was inserted for continuous hemodynamic monitoring as well as arterial blood sampling. Respirations were monitored with a pneumograph.

Microelectrodes were inserted in the peroneal nerve through an access door on the LBNP chamber, and peroneal nerve traffic was recorded from the right lower extremity. Strain-gauge plethysmography and laser Doppler velocimetric blood flows were measured in the right forearm. After complete instrumentation, the subjects rested quietly for 30 minutes. Simultaneous arterial and right forearm venous blood samples were obtained for determination of baseline plasma NE and to provide plasma for determination of [3H]NE recovery by alumina (Al2O3) adsorption. The subjects received an initial bolus of [3H]NE (15 μCi·m-2) over 5 minutes, followed by constant infusion of 0.35 μCi·min-1·m-2 at an infusion rate of 0.2 ml/min for 20 minutes. Thirteen minutes into the infusion, a wrist cuff was inflated to suprasystolic levels. At 14 minutes, skin blood flow measurements were recorded continuously for 1 minute. Blood samples were obtained from both arterial and venous sites at 15 and 20 minutes of the initial infusion period. Muscle sympathetic nerve activity (MSNA) and forearm blood flow were measured during the 5-minute interval between 15 and 20 minutes of constant [3H]NE infusion. MSNA was recorded continuously while eight baseline plethysmographic flow tracings were obtained during the 5-minute period. All flows and venous blood samples were obtained with the wrist occlusion cuff inflated.

Following the initial 20 minutes of continuous infusion, LBNP to −15 mm Hg was initiated. Blood samples were obtained at 5 and 10 minutes (EARLY) and again at 25 and 30 minutes (LATE) of −15 mm Hg LBNP. Skin blood flow was measured at 4 minutes and 24 minutes of −15 mm Hg. MSNA and forearm blood flow (eight curves with wrist occluded) were measured between 5 and 10 (EARLY) and 25 and 30 minutes (LATE). Recovery data were obtained at 10 minutes of recovery in the same sequence as previous measurements.

Lower Body Negative Pressure Technique

Isolated cardiopulmonary baroreceptor unloading was accomplished by exerting −15 mm Hg negative pressure on the lower body while each subject was enclosed in an airtight chamber. Pressure within the chamber was carefully monitored with a standard Statham pressure transducer (American Edwards Laboratories, Irvine, Calif.). It has been previously demonstrated that LBNP of −15 mm Hg produces a decrease in central venous pressure without associated alterations in mean arterial pressure, aortic pulse pressure, or heart rate, thereby avoiding significant activation of arterial (carotid and aortic) baroreflexes.
Preparation and Analysis of Norepinephrine and Tritiated Norepinephrine

Tritiated NE was prepared for each subject just before use by diluting sterile pyrogen-free L-[ring-2,5,6-3H]NE (Du Pont, New England Nuclear, Boston, Massachusetts) of high specific activity (0.25 mCi in 0.2N acetic acid/ethanol [9:1]) in 0.45% saline containing ascorbic acid (2 mg/ml).

After acquisition, blood samples were placed in precooled tubes with 1/50 vol of a solution containing 90 mg/ml EGTA and 60 mg/ml reduced glutathione. The tubes were immediately chilled and centrifuged, and the plasma was stored in aliquots and frozen for later analysis.

Plasma NE concentration was determined by high-performance liquid chromatography (HPLC) with electrochemical detection following alumina adsorption and extraction with perchloric acid. All plasma samples for each subject were run the same day in duplicate. Duplicate extracted samples with known concentrations of NE and the internal standard dihydroxybenzylamine (DHBA) were run simultaneously for determination of standard recoveries. To 4-ml filtration extraction columns were added 100 μl water to saturate the filter, 0.5 or 1.0 ml plasma, acid-activated alumina (20 mg), 100 μl DHBA (5 pg/μl), and 1 ml Tris buffer (1.5 M, pH 8.7). The columns were rotated mechanically for 20 minutes and then placed in a vacuum manifold, and the fluid was aspirated through the filter. After the alumina was washed three times with water (3 ml/wash), the columns were removed from the manifold and centrifuged to remove excess water. After centrifugation, 140 μl of 0.1 M perchloric acid was added to the alumina pellet. This was agitated in a vortex mixer for 20 minutes and centrifuged, and the perchloric acid was collected in 300-μl vials suitable for use in a Kontron Model MSI 600T autosampler (Zurich, Switzerland) with a 50-μl sample loop.

NE and DHBA were separated by reverse-phase HPLC using an 8-cm ESA HR-80 column (Bedford, Massachusetts) packed with 3-μm spherical octadecylsilane. Mobile phase was delivered at 1.5 ml/min by an ESA Model 5700 solvent delivery module and contained the following in one liter: methanol (30 ml), 1-heptane sulfonic acid (0.25 g), Na2EDTA (0.09 g), and monobasic sodium phosphate (6.9 g), adjusted to pH 3.2. NE and DHBA were measured coulometrically using a series of three ESA conditioning/detector cells (models 5021 and 5011) set at the following potentials: +0.35, +0.10, and −0.26 V. Peak heights were determined by a Spectra-Physics Model SP 4270 integrator (San Jose, California).

The [3H]NE concentration was determined by alumina adsorption as described previously.7,8 To 4 ml of plasma was added alumina (200 mg) and 4 ml of 0.5 M ice-cold Tris buffer (pH 8.6) containing 2% Na2EDTA. The suspension was agitation for 10 minutes and then centrifuged, and the supernatant was aspirated. The alumina pellet was washed three times with 10 ml water, and the [3H]NE was extracted with 2 ml of 0.1 M perchloric acid, a sample of which was added to scintillation vials containing 10 ml of scintillation cocktail (ACS II, Amersham, Arlington Heights, Illinois). Samples were counted in a Beckman Model 6800 liquid scintillation spectrometer (Fullerton, California) after dark adaptation. Quench correction was by H number.

A portion of the [3H]NE infusate was frozen at the end of each study and stored for analysis at the time the plasma alumina procedure was performed. The infusate sample was used to determine the [3H]NE specific radioactivity (disintegrations per minute, dpm). To determine the [3H]NE recovery during the alumina extraction, a portion of the infusate was added to duplicate 4-ml baseline plasma samples that were extracted and counted.

Norepinephrine Kinetic Calculations

NE clearance (liters per minute per square meter) and spillover (nanomoles per minute per square meter) were determined by a modification of the technique described by Esler et al.9,10 in which a constant infusion of [3H]NE was administered until steady state was achieved. At steady state, the inference was made that NE clearance is equal to the steady-state [3H]NE infusion rate divided by the actual plasma [3H]NE concentration. Data for clearance and spillover are corrected for the body surface area as described previously7,9,10:

\[
\text{NE clearance} = \frac{[3H]NE \text{ infusion rate (dpm·min}^{-1}·m^{-2})}{\text{plasma [3H]NE (dpm·l}^{-1})}
\]

Plasma NE spillover was then calculated from the [3H]NE infusion rate and the plasma [3H]NE specific activity (dpm·nmol\(^{-1}\)):

\[
\text{NE spillover} = \frac{[3H]NE \text{ infusion rate (dpm·min}^{-1}·m^{-2})}{[3H]NE \text{ specific activity (dpm·nmol}^{-1})}
\]

Although it is assumed that NE release occurs from sympathetic nerve terminals, the contribution to the overall pool for our purposes was assumed to be negligible.

Forearm Blood Flow Technique

Forearm blood flows (milliliters per minute per 100 milliliters of tissue) were measured by the venous occlusion technique using a mercury-in-Silastic single-strand strain-gauge plethysmograph.11–13 This technique measures both muscle and skin blood flow.11 Subjects were supine with the elbow and wrist supported and midforearm free at a height of 10 cm above the heart. After being externally calibrated at a force of 10 g, the strain gauge was placed 10 cm below the olecranon process on the forearm from which venous blood samples would be obtained. Before obtaining any flow measurements, the hand circulation was occluded for 1 minute by inflating to 240 mm Hg a pneumatic cuff placed at the wrist.11 All measure-
ments were performed with a collecting pressure of 50 mm Hg.\textsuperscript{11,13} Arterial blood pressure was recorded simultaneously with each blood flow measurement. Mean arterial blood pressure (mm Hg) was continuously measured with a standard pressure transducer. Forearm resistance was derived from the mean arterial blood pressure and the forearm blood flow. Resistances are expressed in millimeters of mercury per milliliter per minute per 100 milliliters of tissue.

\textit{Laser Doppler Velocimetric Skin Blood Flow Technique}

These measurements were performed to assess the contribution of skin blood flow to overall forearm blood flow changes during LBNP of \(-15 \text{ mm Hg}\). Skin blood flow was measured by the laser Doppler velocimetric technique\textsuperscript{13,14} using a commercially available laser Doppler flowmeter (Laser flow BPM 403, TSI, St. Paul, Minnesota), which was placed 1–2 cm below the mercury-in-Silastic strain-gauge plethysmograph. The technique for acquisition of skin blood flow by this method has been previously described by our laboratory.\textsuperscript{13} With this system, the product of derived blood volume and red cell velocity is used to calculate a number that is proportional to blood flow. The voltage measurement is then multiplied by a conversion factor (6.08) that permits expression of blood flow in milliliters per minute per 100 milliliters of tissue.

\textit{Muscle Sympathetic Neural Activity Measurement Technique}

Multiunit recordings of postganglionic MSNA were measured in each subject through the use of an insulated 200-\(\mu\text{m}\)-diameter tungsten microelectrode that tapered to an uninsulated 1–5-\(\mu\text{m}\)-diameter tip.\textsuperscript{15–17} The microelectrode was inserted transcutaneously into the right peroneal nerve posterior to the fibular head. A reference electrode was inserted subcutaneously 1–3 cm from the recording microelectrode. Neuronal activity was amplified 1,000 times by a preamplifier and 50–100 times by an amplifier. The signal was filtered through a band-pass filter with a bandwidth of 700–2,000 Hz. The filtered signal was rectified and integrated to obtain a mean voltage neurogram. The neurogram was analyzed by manually counting the number of bursts per minute and the total burst amplitude per minute. These parameters have been reported to correlate directly with changes in sympathetic nervous system activity.\textsuperscript{4,15} Criteria for acceptance of a muscle fascicle recording site for analysis during the study were those previously reported by Valbo et al.\textsuperscript{16} These include the following: 1) electrical stimulation (1–4 V, 0.2 msec, 1 Hz) through the recording electrode produced muscle twitches but not paresthesias; 2) the receptive area of the muscle afferents could be plotted by tapping or stretching muscles or tendons but not by lightly stroking the skin that is innervated by the peroneal nerve; and 3) the filtered neurogram revealed spontaneous, pulse-synchronous bursts that increased during prolonged exhalation but not during arousal stimuli (i.e., loud noise or question answering).

\textit{Statistics and Data Analysis}

The baseline NE kinetic data (BASE) represent the average of the 15- and 20-minute resting period blood samples. EARLY and LATE NE data are averages of the 5- and 10-minute and 25- and 30-minute blood samples, respectively. Recovery (REC) NE data represent the blood sample value obtained 10 minutes after termination of LBNP. MSNA at BASE, EARLY, LATE, and REC represents accumulated averages of burst frequency and amplitude per minute obtained over the 5-minute measurement periods. Comparisons of BASE, EARLY, LATE, and REC forearm blood flow data represent an average of eight flow curves obtained during each of the 5-minute measurement periods. Skin blood flow at BASE, EARLY, LATE, and REC each represents 1 minute of continuous averaged laser velocimetric flow measurement.

Statistical analyses of data were made using a within-subject one-way analysis of variance for repeated measures.\textsuperscript{18} If a significant \(F\) value was found, determination of significance between measurement time periods was accomplished using the Student-Newman-Keuls post hoc analysis.\textsuperscript{19} All data are expressed as mean±SEM.

\textit{Results}

The complete results of this study are shown in Table 1. Adequate high-fidelity nerve recordings were obtained in six of the eight subjects studied.

We continuously monitored left radial artery pulse pressure, mean arterial pressure, and heart rate. We noted a small but significant increase in heart rate in response to LBNP (\(F=3.66\ [3/21]\); \(p<0.05\)); however, we found no change in mean arterial blood pressure or pulse pressure (Table 1).

Both MSNA bursts and total amplitude increased significantly during LBNP of \(-15 \text{ mm Hg}\). Total bursts increased from BASE to EARLY and BASE to LATE by 35% and 40%, respectively (\(F=37.34\ [3/15]\); \(p<0.05\)). Total amplitude increased from BASE to EARLY and BASE to LATE by 44% and 53%, respectively (\(F=5.98\ [3/15]\); \(p<0.01\)).

Systemic venous NE spillover increased significantly from BASE to EARLY and BASE to LATE by 17% and 18%, respectively (\(F=20.99\ [3/21]\); \(p<0.05\)) (Figure 1). Arterial NE spillover, although demonstrating an increasing trend, did not change significantly (\(F=2.04\ [3/21]\)) (Table 1). Interestingly, arterial NE clearance decreased significantly from BASE to EARLY by 10%, and from BASE to LATE by 18% (\(F=5.03\ [3/21]\); \(p<0.05\)). Venous clearance, however, did not change significantly (\(F=2.71\ [3/21]\)) (Figure 1).

Forearm blood flow decreased from BASE to EARLY and BASE to LATE by 28% and 26%, respectively (\(F=4.84\ [3/21]\); \(p<0.05\)) (Figure 2). In contrast to the significant decrease in plethysmo-
TABLE 1. Results of the Study

<table>
<thead>
<tr>
<th></th>
<th>BASE</th>
<th>EARLY</th>
<th>LATE</th>
<th>REC</th>
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<tr>
<td>Norepinephrine kinetics data (n=8)</td>
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<tr>
<td>Arterial norepinephrine (pg/ml)</td>
<td>203±36</td>
<td>271±40*</td>
<td>264±44*</td>
<td>240±35*</td>
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<td>Venous norepinephrine (pg/ml)</td>
<td>246±30</td>
<td>325±43*</td>
<td>333±52*</td>
<td>235±31</td>
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<td>Arterial deconstructions per minute</td>
<td>2,165±184</td>
<td>2,383±192†</td>
<td>2,571±185†</td>
<td>2,426±236†</td>
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<td>Venous deconstructions per minute</td>
<td>883±78</td>
<td>904±37</td>
<td>964±76</td>
<td>1,036±84</td>
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<td>Arterial clearance (l/min/m²)</td>
<td>1.08±0.08</td>
<td>0.97±0.06†</td>
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<td>Venous clearance (l/min/m²)</td>
<td>2.72±0.19</td>
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<td>Arterial spillover (nmol/min/m²)</td>
<td>1.25±0.20</td>
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<td>Venous spillover (nmol/min/m²)</td>
<td>4.08±0.71</td>
<td>4.92±0.58†</td>
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<td>3.23±0.52</td>
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<td>Muscle sympathetic neural activity (n=6)</td>
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<td>Bursts/min</td>
<td>24±3.2</td>
<td>37±2.7†</td>
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<td>Mean burst amplitude (mm)</td>
<td>346±77</td>
<td>623±145*</td>
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<td>Hemodynamic parameters (n=8)</td>
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<td>Mean arterial pressure (mm Hg)</td>
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<td>Heart rate (beats/min)</td>
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<td>62±3†</td>
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<td>Forearm plethysmographic data (n=8)</td>
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<td>Blood flow (ml/min/100 ml)</td>
<td>4.5±0.6</td>
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<td>Resistance (mm Hg/ml/min/100 ml)</td>
<td>23±2.7</td>
<td>31±3.7†</td>
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<td>Laser doppler skin blood flow data (n=8)</td>
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<tr>
<td>Blood flow (ml/min/100 ml)</td>
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<td>Resistance (mm Hg/ml/min/100 ml)</td>
<td>60±11</td>
<td>76±23</td>
<td>64±17</td>
<td>67±12</td>
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Values expressed as mean±SEM, which represents the standard error of the individual mean values presented. BASE, measurements made at baseline; EARLY, at 5 and 10 minutes; LATE, at 25 and 30 minutes; REC, at 10 minutes of recovery during continuous lower body negative pressure.

*p<0.05; †p<0.01; indicate significance between each time period and baseline values as determined by Student-Newman-Keuls post hoc analysis, after determination of significant F values by one-way analysis of variance for repeated measures.

Graphically obtained measures of forearm blood flow, skin blood flow did not change (F=1.41 [3/21]) (Figure 2).

The patterns of change for plasma NE spillover, MSNA, and forearm vascular resistance are shown in Figure 3. The patterns of activation for these separate indexes of neural activity are strikingly similar. Of note, we found that no index of increased neural activity demonstrated significant differences between EARLY and LATE values, indicating probable achievement of a steady state during the EARLY phase of LBNP.

Plasma NE concentrations increased significantly to similar levels in the arterial (F=7.63 [3/21]; p<0.01) and forearm venous circulations (F=21.23
Percent change in muscle sympathetic nerve activity (MSNA) total amplitude and total bursts, forearm vascular resistance, and venous norepinephrine spillover measured at baseline (BASE), at 5 and 10 minutes (EARLY), at 25 and 30 minutes (LATE), and at recovery (REC) during continuous lower body negative pressure at -15 mm Hg pressure.

[3/21]; \( p<0.01 \) (Table 1). However, as demonstrated in Figure 4, the relative contributions of NE spillover and clearance in each circulatory bed were quite different. In the arterial circulation, clearance was the primary process causing the increase in plasma NE concentration. Although there was a trend toward an increase in arterial spillover EARLY, this increase did not achieve statistical significance and was not evident LATE. In contrast, venous plasma drawn from the forearm revealed that spillover was the more important mechanism causing the increase in plasma venous NE.

Five of the six subjects in which both MSNA and forearm venous NE spillover were measured showed larger percent increases in MSNA when compared with NE spillover. If the various parameters are compared within individuals, however, substantial variability is noted. For example, the subject with the greatest variability showed a 74% increase in MSNA total amplitude and a 20% reduction in forearm venous NE spillover along with an 8% reduction in forearm venous NE concentration. This was the only subject with a decrease in NE forearm venous spillover and NE concentration. This subject had a small increase in plasmohymographically determined forearm vascular resistance (18%) (reflecting skin and muscle vascular resistance) but a large reduction in skin vascular resistance (26%).

Radioactive counts obtained in studies of five subjects during supine, 80-minute constant infusion of [3H]NE revealed no significant change between each 20-minute sample when compared with the sample obtained 20 minutes after completing the bolus, as determined by one-way analysis of variance for repeated measures \( (F=0.927) \). These data demonstrate that the reductions in NE clearance noted during the primary study were not due to the inability to achieve a steady state but rather were a true reflection of a reduction in arterial NE clearance.

An example of original and filtered peroneal nerve traffic, electrocardiogram, arterial pressure, and forearm blood flow from one subject is presented in Figure 5.

**Discussion**

In this study we investigated the effects of prolonged isolated cardiopulmonary baroreceptor

**Figure 3**. Percent change in muscle sympathetic nerve activity (MSNA) total amplitude and total bursts, forearm vascular resistance, and venous norepinephrine spillover measured at baseline (BASE), at 5 and 10 minutes (EARLY), at 25 and 30 minutes (LATE), and at recovery (REC) during continuous lower body negative pressure at -15 mm Hg pressure.

**Figure 4**. Percent change in systemic plasma arterial and forearm venous norepinephrine (NE) concentrations, systemic arterial and forearm venous NE clearance, and systemic arterial and forearm venous NE spillover at 25 and 30 minutes compared with baseline during continuous lower body negative pressure at -15 mm Hg pressure.

**Figure 5**. Representative tracings from one subject obtained at baseline (BASE) and at 5 and 10 minutes (EARLY). Panel A: electrocardiogram; panel B: arterial pressure; panel C: filtered peroneal nerve traffic; panel D: original peroneal nerve tracings; panel E: forearm blood flow. Of note, lower body negative pressure caused a change in both plethysmographic forearm blood flow and nerve traffic, without significant change in mean arterial pressure or pulse pressure.
unloading on the sympathetic nervous system efferent limb response. Using prolonged nonhypotensive LBNP of −15 mm Hg, we examined changes in nerve traffic, NE kinetics, and forearm and skin blood flow.

The principle findings of our study were that during −15 mm Hg LBNP 1) nerve traffic to skeletal muscle increased significantly; 2) venous NE spillover measured in an antecubital vein of the forearm increased significantly, whereas arterial NE spillover did not change; 3) arterial clearance decreased significantly, whereas venous clearance did not; 4) plethysmographic measurements of forearm blood flow decreased significantly but skin blood flow remained constant; and 5) the percent increases in arterial and venous NE concentrations were similar although the mechanisms for the increase in NE concentrations in each circulation appeared to be different. We believe these findings are consistent with the concept that unloading of cardiopulmonary baroreceptors leads to increased nerve traffic predominantly to muscle and causes a reduction in systemic arterial NE clearance that serves to increase systemic arterial NE concentrations. The following discussion addresses each of the principle findings and their potential implications.

**Sympathetic Efferent Nerve Activity**

We have demonstrated increases in peroneal nerve MSNA during exposure to −15 mm Hg LBNP in normal subjects. The increase of limb postganglionic nerve traffic in response to isolated cardiopulmonary baroreceptor unloading has been described previously by Sundlof and Wallin,4 who demonstrated increases in median nerve burst frequency and amplitude. Victor and Leimbach,3 studying MSNA in the peroneal nerve of the leg, have shown similar increases in nerve traffic. Rea and Wallin5 also have demonstrated similar increases in nerve traffic in both the radial and peroneal nerves in response to LBNP. Our findings expand on these observations by demonstrating the relation between measures of junctional NE release and postganglionic nerve traffic in humans.

### Norepinephrine Spillover and Clearance:

**Arterial and Venous**

Spillover of NE to plasma represents 22% of total body NE released.20 Despite this limitation, a change in NE spillover is a more accurate indicator of sympathetic nervous system activation than circulating plasma NE because plasma NE reflects alterations in both NE spillover and NE clearance.6,7 Measurement of venous NE spillover in blood obtained from an antecubital forearm vein has been thought to represent NE release and NE extraction predominantly in the forearm.21 In our study, we found that venous NE spillover measured from the forearm increased significantly but to a lesser degree than that of peroneal nerve traffic. This finding may reflect alterations in the junctional handling of NE and can potentially be explained by increased NE reuptake, increased metabolic degradation of NE, or increased α-receptor inhibition of NE junctional release. These potential mechanisms may result in a nonlinear relation between muscle sympathetic nerve traffic in the peroneal nerve and NE spillover measured from forearm venous blood. Another potential explanation is that a dilutional effect occurs by admixture of blood from sympathetic nervous system–activated muscle vasculature, with blood from the inactive cutaneous vasculature of the forearm. This is perhaps the most likely explanation; indeed, data of one subject presented in our results strongly support this concept. In this subject, MSNA increased by 74%, whereas NE spillover and NE concentration decreased. Not surprisingly, this subject had a 33% increase in forearm skin blood flow. Parenthetically, we believe that it is this variable dilutional effect by skin blood flow that makes intra-individual comparisons of MSNA and forearm venous NE spillover difficult. Finally, it should be stated that MSNA was measured in the leg and venous spillover was measured in the forearm. This also could potentially cause different percent changes from baseline. We think this is unlikely because Rea and Wallin5 have recently shown similar percent changes from baseline for peroneal and radial nerve MSNA in response to baroreceptor unloading.

Unlike forearm venous spillover, no significant change was found in arterial spillover. This suggests there is less activation of systemic beds aside from muscle. This may in part account for the overall higher concentration of both arterial and venous plasma NE in response to hypotensive LBNP compared with concentrations noted with lower levels of LBNP. Hypotensive LBNP presumably unloads both low- and high-pressure baroreceptors and results in greater plasma NE concentrations than with cardiopulmonary baroreceptor unloading alone.7,22

A surprising finding of the present study was the difference in NE spillover and clearance in the forearm venous and arterial circulations. Our data suggest that during low-pressure baroreceptor unloading, skeletal muscle NE spillover is the predominant mechanism that raises forearm venous plasma NE concentrations. In contrast, in the arterial circulation, the increase in plasma NE is more influenced by changes in arterial NE clearance. The mechanism for this reduction in arterial NE clearance is unclear, although it is known that LBNP and orthostatic stress can reduce cardiac output and alter the distribution of blood flow.22-24; both of these factors can reduce NE clearance.7 Thus, we postulate that low-pressure baroreceptor unloading exerts its predominant effect on the concentration of arterial NE by reducing cardiac output only, by altering the distribution of blood flow, or both. Further studies are necessary to confirm this postulate.

### Plethysmographic and Laser Doppler Blood Flow

Blood flow responses to sympathetic nervous system activation have been studied by several
Limitations

Both skin and muscle vascular resistance changes occur in response to baroreceptor unloading induced by LBNP. It should be noted, however, that the skin resistance changes reported in that study were the result of combined low- and high-pressure baroreceptor unloading with 60 mm Hg LBNP for 3 minutes. Our observations regarding the lack of significant changes in skin blood flow are consistent with previous work demonstrating that skin blood flow does not change significantly during LBNP of less than −20 mm Hg. Thus, we believe our findings are consistent with the postulate that the overwhelming decrease in forearm blood flow that occurs with −15 mm Hg LBNP is due to decreases in muscle blood flow.

Potential Limitations

Because of minor but significant increases in heart rate noted in our subjects in response to LBNP at −15 mm Hg, there may have been some degree of arterial as well as cardiopulmonary baroreceptor unloading. We believe this effect on arterial baroreceptors was minimal in light of absent changes in mean arterial pressure and aortic pulse pressure. In addition, the plasma NE concentrations found in our subjects were only a fraction of those previously reported when normal subjects were subjected to head-up tilt, a stimulus known to unload both cardiopulmonary and arterial baroreceptors.

Prior reports have used calculations of regional NE kinetics to determine the contribution of various organ vascular beds to overall systemic NE kinetics. Although determination of regional NE kinetics of the forearm might have been useful in quantifying NE extraction and in measuring local NE release and clearance more accurately, calculations were not performed in our subjects because of our inability to partition muscle blood flow from that of skin blood flow. Without knowing forearm muscle mass, muscle blood flow could not be calculated. Moreover, the antecubital blood sample was composed of both skin and muscle effluents so we could not partition these components and had no way of knowing exactly how LBNP would affect the relative contribution from each vascular bed. This last factor is especially important in our study because we found no significant difference in laser Doppler velocimetrically determined skin blood flow in response to low levels of LBNP. Therefore, we elected to discuss only the previously calculated parameters of venous clearance and spillover.

Conclusions

This study represents the first report using a direct nerve recording technique and continuous-infusion [3H]NE radiotracers methodology simultaneously to study cardiopulmonary baroreceptor function in humans. Using these modalities in conjunction with forearm plethysmography and laser Doppler velocimetry, we have demonstrated that low-pressure baroreceptor unloading predominately activates skeletal muscle. Moreover, our data suggest that reduced clearance of NE in the arterial circulation is the primary mechanism by which arterial NE concentration rises, whereas increased forearm venous NE spillover is predominantly responsible for increased venous NE concentrations noted during isolated cardiopulmonary baroreceptor unloading.

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References


**KEY WORDS** • norepinephrine kinetics • microneurography • lower body negative pressure • cardiopulmonary baroreceptors • forearm skin and muscle blood flow
Direct neurohumoral evidence for isolated sympathetic nervous system activation to skeletal muscle in response to cardiopulmonary baroreceptor unloading.

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