Effects of graded LBNP on MSNA and interstitial norepinephrine

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**Experimental paradigm.** Subjects reported to the General Clinical Research Center in the morning after an overnight fast. They were instructed to refrain from caffeine intake for 24 h before the experiment. Subjects were placed on a padded table with their lower body (up to the level of the umbilicus) positioned inside a LBNP chamber. An IV was placed in an antecubital vein. To perform vastus lateralis muscle microdialysis, a LBNP chamber was constructed in which only the left leg and pelvis were placed within the tank and exposed to LBNP. The right leg from the level of upper thigh down remained outside the tank and readily accessible for the performance of microdialysis and microneurography. Continuous monitoring of the heart rate (HR) was performed with ECG, and arterial blood pressure (BP) was measured using the volume-clamp method (Finapres, Ohmeda; Madison, WI). Microdialysis probes and the peroneal nerve electrodes were then placed (see below for details).

After instrumentation and a 90-min “equalization period,” baseline MSNA, BP, and HR were recorded and microdialysate and blood samples were collected. After 10 min of baseline, LBNP was initiated. The negative pressure was applied in a graded incremental manner beginning with −10 mmHg, proceeding to −30 mmHg, and then to −50 mmHg. All stages were 10 min in length, and BP and HR were closely moni-
tered during this time. The experiment was terminated when
the paradigm was completed or if any of the following oc-
curred: 1) volunteers developed presyncopal symptoms such
as lightheadedness, dizziness, nausea, cold sweats, yawning,
or visual disturbances, 2) volunteers developed a sudden fall
in systolic BP of $> 35$ mmHg, and/or 3) volunteers developed
a fall in systolic BP to $< 85$ mmHg.

Microdialysis. The microdialysis probes were inserted in
the vastus lateralis muscle of the subject’s right leg. This
muscle was chosen because there are no important nerves
or vessels passing through it and thus the risk of neurovascular
damage to the lower extremity was extremely small. An area
measuring $25 \times 20$ cm over the subject’s thigh was shaved,
prepped with povidone-iodine solution, and then draped us-
ing sterile sheets. A skin marker was used to mark the probe
insertion sites, which were $2 - 3$ cm apart. With the use of
strict aseptic techniques, the skin and subcutaneous sites at
the probe entry and exit sites were anesthetized with a local
injection of lidocaine (0.5–1.0 ml). The probes, four to six
in number (depending on the muscle size and orientation),
were inserted into the muscle via a 20-gauge cannula. After
insertion, the microdialysis probes were attached to a perfusion
pump (CMA model 102) and perfused at a rate of 5 $\mu$l/min
with a saline solution containing 3.0 mM glucose and 0.5 mM
lactate.

Microdialysis probes. The semipermeable fibers (GFS Plus
12, Gambro) used to construct the microdialysis probes had a
molecular mass cutoff of 3,000 kDa [0.20 mm inner diameter
(ID), 0.22 mm outer diameter (OD)]. Briefly, each end of a
single fiber was inserted ~1 cm into a hollow polyamide tube
(0.25 mm ID, 0.36 mm OD) and glued, and the actual diffus-
ible portion (distance between the two polyamide tubes) was
4 cm. A complete description of microdialysis probe construc-
ction can be reviewed in MacLean et al. (19).

Determination of probe recovery. To fully utilize the micro-
dialysis technique, an estimate of the extraction fraction of
the compound being measured in the interstitial space needs
to be made, which is defined as “probe recovery.” This deter-
mination is necessary to calculate actual interstitial concen-
trations and to document any possible changes that may
occur to probe recovery during the course of the experiment.
To achieve this, a small amount of radioactive tracer, in the
form of compound being investigated, is added to the micro-
dialysis perfusate. It has been suggested that the relative
loss of the isotope from the perfusate into the interstitial
space represents probe recovery for that compound. This was
confirmed in vitro by Kurosawa et al. (15), where the simul-
taneous measurement of tracer loss and compound recovery
proved to be similar. The major advantage of this method is
that probe recovery can be determined in vivo for each col-
clected sample allowing continuous monitoring of probe recov-
ery over time.

To determine the probe recovery of NE in this study, a
small amount of $[^{3}H]$NE (0.1 $\mu$Ci/ml) was added to the per-
fusate. Probe recovery was calculated from the loss of radio-
active tracer, and the actual interstitial concentration of NE
was calculated from the probe recovery values and dialysate
NE concentrations (18).

Microneurography. This technique provides direct record-
ings of sympathetic nerve activity directed to blood vessels in
skeletal muscle. MSNA was our primary index of sympa-
thetic activity. External mapping of the peroneal nerve course
(40–100 V, 0.2 ms, 1 Hz) was performed just behind the
fibular head of the right leg. A reference electrode was placed
subcutaneously $2 - 3$ cm away from the recording site. Mul-
tiunit recordings of MSNA in the leg were obtained by trans-
cutaneous placement of a tungsten microelectrode (insulated
200 $\mu$m diameter with a tapered uninsulated 1- to 5-$\mu$m tip)
into a C-fiber-containing fascicle in the nerve. The electrode
was manipulated to yield a neurogram with characteristic
bursts of MSNA. The signals thus obtained represent post-
ganglionic vasoconstrictor nerve traffic. The signals were
amplified 1,000 times by a preamplifier and 50–100 times by
an amplifier. The signals were filtered (bandwidth of 700–
2,000 Hz), rectified, and integrated to obtain a mean voltage
equivalent of the mean MSNA burst waveform whether the cycle
contained a burst or not. It then divided this composite by the
number of cycles in the original data segment to yield the aver-
age MSNA signal per cycle, which was then numerically inte-
grated to determine the area under the curve.

Blood and dialysate samples. A Teflon intravenous cathe-
ter was placed in a forearm vein and attached to a saline lock
to ensure patency. Approximately 3 ml of blood were drawn
during baseline and then during minute 9 of each level of
LBNP. Blood samples were placed in prechilled tubes con-
taining EDTA and glutathione and immediately centrifuged,
and the plasma was separated and frozen at $-80^\circ$C until
analyzed. One milliliter of plasma was used for the determi-
nation of NE concentrations (100 $\mu$l injection volume) using
HPLC and electrochemical detection (26). The specific activity of $[^{3}H]$NE in 5 $\mu$l of collected dialysate was deter-
mined using traditional liquid scintillation counting tech-
iques. Dialysate samples were collected with 2 $\mu$l of a
3 mM sodium metabisulfite solution and did not require
any further processing due to the highly filtered nature of
the samples. Forty microliters of dialysate were combined with
60 $\mu$l of 0.1 M glacial acetic acid (75 $\mu$l injection volume),
and NE concentrations were determined by HPLC in a similar
fashion to the plasma samples.

MSNA was analyzed as bursts. It was measured during baseline
and at minute 9 of each 10-min level of LBNP ($-10$,
$-30$, and $-50$ mmHg). HR and mean arterial pressure
(MAP) were also used for comparison of the different LBNP
stages (see below).

Signal-averaged MSNA was measured each minute for
every level of LBNP. With the use of a paired $t$-test, we
found that for each level of LBNP, minute 9 was not statisti-
cally different than any other minute. HR and MAP values at
minute 9 were also compared with each other minute. We
found that at $-30$ and $-50$ mmHg, HR took 4 and 3 min,
respectively, to reach a steady-state value. At $-50$ mmHg,
the minute 2 value for MAP was 3.3 mmHg higher than the
minute 9 value (91.9 $\pm$ 3.9 mmHg at minute 9 and 95.2 $\pm$ 3.9
mmHg at minute 2, $P < 0.036$). Thus all values reached a
steady state by minute 9.

Microdialysate was collected over the entire 10-min base-
line and LBNP periods to obtain a sufficient volume for NE
analysis. The signals for BF, ECG, and MSNA were sampled
at 100 Hz and stored on a computer using a commercially
available data-acquisition system (PowerLab, ADInstru-
ments).

Statistical analysis. We analyzed MAP, HR, MSNA, and
NEp using a two-way ANOVA. Two main effects were exam-
ined: LBNP and at each level of LBNP. A signal-averaged MSNA wave-
form was produced for each segment using the signal-aver-
aged ECG (SAECG) extension within the PowerLab v.4.1
software package (ADInstruments). SAECG used the ECG to
trace the MSNA signal into packets spanning individual
cardiac cycles and summed all MSNA cycles into a composite
waveform whether the cycle contained a burst or not. It then
divided this composite by the number of cycles in the original
data segment to yield the average MSNA signal per cycle,
which was then numerically integrated to determine the area
under the curve.

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analysis. The signals for BF, ECG, and MSNA were sampled
at 100 Hz and stored on a computer using a commercially
available data-acquisition system (PowerLab, ADInstru-
ments).

Statistical analysis. We analyzed MAP, HR, MSNA, and
NEp using a two-way ANOVA. Two main effects were exam-
ined: LBNP (a within-subject variable with 4 levels: baseline,
−10, −30, and −50 mmHg) and probe placement (a between-subject variable with 2 levels: subjects with and without microdialysis probes). This approach allowed us to determine whether 1) the one-legged LBNP tank that was specially designed for these experiments evoked graded autonomic responses and 2) microdialysis probe placement altered the autonomic responses. NEi was analyzed with a one-way ANOVA with repeated measures for the four levels of LBNP. All post hoc analyses were performed using Dunnett’s test to compare all values to baseline.

To test the relationship between NE and MSNA, we performed a Pearson’s partial correlation, which was adjusted for the paradigm (1). This approach was used to account for the four levels of LBNP.

NEi data were collected from a total of 15 microdialysis probes in 6 subjects; however, before statistical analysis, these data were averaged within each subject so that each subject had only one NEi value for each level of LBNP. All data are expressed as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Effects of microdialysis probe insertion on measured hemodynamic variables. As can be seen in Table 1 (n = 12), there was no effect of probe insertion on HR, MAP, NEp, or MSNA (“probe” main effects were not significant for all 4 variables). Thus the act of measuring NEi did not in itself systematically alter the study of LBNP effects on hemodynamics.

Effects of one-legged LBNP on HR, MAP, NEp, and MSNA. As expected, LBNP led to an increase in HR (main effect P < 0.031) and MAP (main effect P < 0.046). Of note, both NEp and MSNA bursts rose with LBNP (main effects P < 0.025 and P < 0.001, respectively). Pointwise comparisons demonstrated statistically significant differences between baseline and LBNP −50 mmHg values for all four parameters.

Effects of one-legged LBNP on NEi. The number of probes used for the determination of NEi at baseline and during −10, −30, and −50 mmHg LBNP were 15, 14, and 10, respectively. The reasons for this difference in the number of probes at different levels of LBNP were as follows: 1) As LBNP increased, some probes ceased to function. LBNP induced leg movement, which caused some probes to kink. This resulted in a reduced volume of perfusate, which was assessed by weighing the sample tubes after collection, and 2) the development of presyncope, which resulted in insufficient dialysate to measure NEi.

The coefficient of variation for the four subjects in whom multiple probes were used to measure NEi was 24.2% for all levels of LBNP. In vivo probe recovery for NE remained unchanged throughout the experiment. Probe recovery during baseline and −10, −30, and −50 mmHg LBNP was 25.1 ± 0.6%, 24.0 ± 0.6%, 24.7 ± 0.6%, and 23.8 ± 0.7%, respectively. LBNP led to a large increase in NEi. The values were 5.2 ± 0.8 nM at rest, 8.4 ± 0.9 nM at −10 mmHg, 11.7 ± 1.5 nM at −30 mmHg, and 17.0 ± 1.7 nM at −50 mmHg (P < 0.001).

Comparative effects of LBNP on NEp and NEi. The percent increase from baseline to the maximum level of LBNP was much greater for NEi than for NEp (n = 6,

Table 1. Data from all levels of the LBNP protocol

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>−10 mmHg</th>
<th>−30 mmHg</th>
<th>−50 mmHg</th>
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<tbody>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With microdialysis</td>
<td>6</td>
<td>57.6 ± 5.6</td>
<td>58.2 ± 5.5</td>
<td>63.5 ± 5.1</td>
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<tr>
<td>Without microdialysis</td>
<td>6</td>
<td>67.0 ± 2.4</td>
<td>69.8 ± 4.4</td>
<td>74.0 ± 4.6</td>
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<tr>
<td>Probe effect</td>
<td></td>
<td>2.09</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>LBNP effect</td>
<td></td>
<td>3.40</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td>1.10</td>
<td>0.367</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With microdialysis</td>
<td>6</td>
<td>91.7 ± 1.7</td>
<td>91.3 ± 2.6</td>
<td>86.5 ± 3.4</td>
</tr>
<tr>
<td>Without microdialysis</td>
<td>6</td>
<td>92.3 ± 2.9</td>
<td>91.5 ± 3.9</td>
<td>92.2 ± 3.4</td>
</tr>
<tr>
<td>Probe effect</td>
<td></td>
<td>1.06</td>
<td>0.327</td>
<td></td>
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<tr>
<td>LBNP effect</td>
<td></td>
<td>3.01</td>
<td>0.046</td>
<td></td>
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<tr>
<td>Interaction</td>
<td></td>
<td>1.72</td>
<td>0.185</td>
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<tr>
<td>NEp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With microdialysis</td>
<td>6</td>
<td>0.95 ± 0.13</td>
<td>1.04 ± 0.16</td>
<td>1.03 ± 0.16</td>
</tr>
<tr>
<td>Without microdialysis</td>
<td>6</td>
<td>0.91 ± 0.08</td>
<td>1.11 ± 0.13</td>
<td>1.33 ± 0.14</td>
</tr>
<tr>
<td>Probe effect</td>
<td></td>
<td>0.81</td>
<td>0.389</td>
<td></td>
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<tr>
<td>LBNP effect</td>
<td></td>
<td>3.66</td>
<td>0.025</td>
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<tr>
<td>Interaction</td>
<td></td>
<td>1.15</td>
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<tr>
<td>MSNA</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>With microdialysis</td>
<td>6</td>
<td>13.7 ± 2.4</td>
<td>16.6 ± 3.4</td>
<td>20.0 ± 4.4</td>
</tr>
<tr>
<td>Without microdialysis</td>
<td>6</td>
<td>20.5 ± 4.7</td>
<td>24.5 ± 3.9</td>
<td>24.0 ± 6.0</td>
</tr>
<tr>
<td>Probe effect</td>
<td></td>
<td>1.39</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>LBNP effect</td>
<td></td>
<td>8.80</td>
<td>0.901</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td>0.48</td>
<td>0.700</td>
<td></td>
</tr>
</tbody>
</table>

Data presented are means ± SE; n = no. of subjects. HR, heart rate (in beats/min); MAP, mean arterial pressure (in mmHg); NEp, plasma norepinephrine (in nM); MSNA, muscle sympathetic nerve activity (in bursts/min); LBNP, lower body negative pressure. Statistics are the results of two-way ANOVA between the six subjects that had microdialysis placed versus the six subjects that did not have the procedure. The results show no effect of the microdialysis procedure on these variables. Values at −50 mmHg are different from baseline for HR, NEp, and MSNA.
197 ± 52% vs. 21 ± 6%, P < 0.015, paired t-test). This led to a progressive NEi-to-NEp gradient (Fig. 2).

MSNA bursts correlated with NEi (Fig. 3; \( r = 0.721, P < 0.004, n = 6 \)). Similar relationships were noted when signal-averaged MSNA was used (\( r = 0.675, P < 0.011, n = 6 \)). A relationship between MSNA bursts and NEp was also noted (\( r = 0.319, P = 0.05, n = 12 \) subjects).

**DISCUSSION**

The main findings of the study are as follows: 1) one-legged LBNP led to a rise in MSNA, NEi, and NEp at −50 mmHg; 2) MSNA correlated with both NEi and NEp; and 3) the rate of rise of NEp as LBNP increased was much less than the rate of increase in NEi. The results of our study suggest that NEi concentrations are reflective of changes in SNS activity seen during baroreceptor unloading. Furthermore, we believe that NE concentrations at the neurovascular junction are better reflected by NEi than by NEp. Finally, the large gradient in NEi and NEp, which increases as a function of LBNP, suggests that the capillary barrier and the level of blood flow contributes importantly to the NEp level.

**Assessment of autonomic function.** Because of the significance of the SNS in circulatory control, numerous methodologies have been employed to understand its functioning under various physiological conditions. These include plasma measurements of NE kinetics and the measurement of MSNA using microneurography. NE kinetics have been studied extensively using compartmental and noncompartmental techniques developed by Linares, Esler, and others (3, 7, 10, 17). The steady-state \(^{3}H\)NE technique is the most commonly used method to measure NE spillover. The major drawback of this technique is that the rate of entry of NE into the circulation during “stress” may or may not represent the same percentage of released NE that is seen under resting conditions. It must be emphasized that aside from NE release, many local and systemic factors can influence NE spillover. The local factors include NE uptake and metabolism, the width of the neuronal cleft, and conductivity of the capillary bed (6). Systemic factors include the level of cardiac output as well as regional blood flow (2, 4). These additional factors render NE spillover an imprecise index of NE released from sympathetic nerve terminals.

Microneurographic recordings of sympathetic nerve traffic represent another method that has been utilized to increase our understanding of autonomic control in human subjects. This technique provides a measure of sympathetic nerve traffic; however, it does not provide direct information regarding the events taking place at the neuroeffector junction. Moreover, it can be argued that with microneurography, sympathetic “activity” is measured in a nerve fascicle that is proximal to the sympathetic nerve ending. Accordingly, it is unclear whether all nerve impulses translate into electrochemical coupling and the release of NE at the neurovascular junction.

Despite these caveats, important information has been gathered by measuring MSNA and NE spillover. For example, in studies conducted by Wallin et al. (25), a positive correlation between MSNA and total body NE spillover and NEp concentration at rest and during isometric hand grip exercise has been shown. Kingwell et al. (14) demonstrated a positive correlation between...
MSNA and cardiac NE spillover at rest. Thus NE spillover under the appropriate circumstance may be reflective of increased SNS activity. We suggest that this relationship may be greatly dependent on the level of regional blood flow as well as the diffusion capacity of NE from the interstitium into the vascular space.

**Study findings and their implications.** In the present report, we used the microdialysis technique to directly determine NE\(_i\) concentrations at rest and during LBNP. With the application of LBNP, the SNS is activated through unloading of baroreceptors leading to an increase in the MSNA (23). In addition, LBNP can be employed in a graded and incremental fashion, leading to graded levels of sympathetic activation (3).

In the present study, LBNP resulted in an increase in MSNA associated with a corresponding increase in NE\(_i\). MSNA correlated with both NE\(_i\) and NE\(_p\). However, the rate of rise in NE\(_p\) was much less than that noted for MSNA vs. NE\(_i\) (Fig. 2). At rest, the NE\(_p\) concentration was ~18% of the NE\(_i\) concentration; at ~50 mmHg LBNP, NE\(_p\) was only 7% of NE\(_i\).

Parenthetically, the increase in NE\(_p\) compared with baseline is smaller than the increases seen in previous reports. We suspect that the magnitude of the increase was less in this report because we used the less vigorous one-legged LBNP as the stimulus and not two-legged LBNP as the stimulus to increase sympathetic outflow (11).

What mechanisms might explain these findings? Previous studies have suggested that the endothelium serves as a barrier to NE diffusion from the interstitium into the circulation (21). We suggest that this barrier accounts for the NE\(_i\)-to-NE\(_p\) blood concentration gradient seen at rest. Furthermore, a decrease in local blood flow can alter the gradient for diffusion, which could increase the NE\(_i\)-to-NE\(_p\) gradient (9). In our studies, LBNP led to an increase in MSNA and NE\(_i\), and presumably to a greater level \(\alpha\)-receptor stimulation by NE. These \(\alpha\)-receptors are located at the metarteriole and precapillary levels. We speculate that this process led to a reduction in the intracapillary pressure, which was already compromised by low limb flows as a result of the diminished cardiac output seen with LBNP. These processes may have even produced a collapse of some capillary beds, which would have resulted in an even greater gradient between NE\(_i\) and NE\(_p\) (5, 20). We hypothesize that this effect reduced the area available for diffusion and limited the rate of NE diffusion into the general circulation (Fig. 2). Further work will be necessary to test this line of reasoning.

It should be noted that a possible limitation to the findings is the different anatomic location from where each of the variables was measured. For example, NE\(_p\) was measured from an antecubital vein, NE\(_i\) was measured from the vastus lateralis muscle, and MSNA was measured from the peroneal nerve. It is possible that the degree of sympathetic outflow at the recording site differed from that at the site of NE\(_i\) measurement. Moreover, it is possible that there was somewhat less release in NE from the nerves in the arm than in the leg for a given level of MSNA. Future studies comparing NE\(_i\) in the leg and arm will be necessary to further explore this issue. Despite these potential drawbacks, the magnitude of change in NE\(_i\) compared with NE\(_p\) was so substantial that any influence of regional MSNA or NE release differences are unlikely to explain the observed findings.

In conclusion, activation of the SNS with LBNP leads to increases in MSNA, NE\(_i\), and NE\(_p\). Both NE\(_i\) and NE\(_p\) correlate with MSNA. At rest, a large concentration gradient exists between NE\(_i\) and NE\(_p\). This gradient increases with LBNP. We believe that our findings suggest that 1) NE\(_i\) is a better reflection of NE release than NE\(_p\), and 2) the capillary diffusion barrier as well as the level of regional blood flow importantly contribute to this gradient.

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**REFERENCES**


