Effects of Lower-Body Negative Pressure on Sympathetic Nerve Responses to Static Exercise in Humans

Microneurographic Evidence Against Cardiac Baroreflex Modulation of the Exercise Pressor Reflex

Urs Scherrer, MD, Susanne Fløistrup Vising, MD, and Ronald G. Victor, MD

Although previous studies in both animals and humans have suggested that cardiac baroreceptors modulate reflex sympathetic vasoconstriction during exercise, more recent studies in conscious animals have not supported this view. To further test this concept in humans, we measured sympathetic nerve discharge with intraneural microelectrodes while we used static handgrip to activate the exercise pressor reflex and nonhypotensive lower-body negative pressure (LBNP) to selectively unload the cardiac baroreflex. In nine healthy subjects, we measured blood pressure, heart rate, central venous pressure, and muscle sympathetic nerve activity (MSNA) from the peroneal nerve (resting leg) during 2 minutes of static handgrip at 20% and 30% of maximal voluntary contraction (MVC) alone and in combination with LBNP at −5 mm Hg. Handgrip alone (exercise reflex) at 20% and 30% MVC caused graded increases in MSNA. LBNP alone (cardiac reflex) did not alter blood pressure or heart rate but decreased central venous pressure by 2.5±0.1 mm Hg (mean±SEM, p<0.05) and increased MSNA by 92±22% over the control value. Most important, handgrip performed during LBNP (interaction of reflexes) caused increases in MSNA that were comparable with the increases during handgrip alone: +114±32% versus +175±89% at 20% MVC and +328±101% versus +431±110% at 30% MVC (handgrip plus LBNP vs. handgrip alone, p>0.1). Pressor and heart rate responses to handgrip also were unaffected by LBNP. In five additional experiments, comparable findings were obtained when the LBNP was superimposed on handgrip rather than handgrip being superimposed on LBNP. In conclusion, this study provides direct evidence in humans that nonhypotensive LBNP does not augment muscle sympathetic outflow during static handgrip and challenges the concept of an important interaction between cardiac baroreceptor and exercise pressor reflexes during this form of exercise. (Circulation 1988;78:49–59)

Several clinical studies have suggested that upright position and orthostatic stress can influence circulatory responses during exercise testing.1–3 For example, in patients with coronary artery disease, increases in arterial pressure, heart rate, cardiac index, and left ventricular stroke work and systolic ejection rate indexes were all greater during upright than during supine bicycle exercise.4,5 These differences have generally been attributed to the effects of upright posture on the loading conditions of the left ventricle (i.e., a mechanical effect), and the possibility that orthostatic stress may also alter autonomic reflex regulation of cardiovascular function in this setting has received relatively little attention.

One reflex mechanism that may be implicated in these differential responses is that the decrease in cardiac filling pressure during upright posture unloads cardiac baroreceptors with vagal afferents.4 This afferent discharge is thought to provide information to the central nervous system of changes in
central blood volume, such that a decrease in afferent impulse traffic signals a decrease in cardiac filling and evokes reflex increases in sympathetic outflow and peripheral vascular resistance. There is increasing experimental evidence that this reflex is a primary factor in producing the reflex autonomic adjustments to orthostatic stress. However, the importance of the cardiac baroreflex in modulating the circulatory responses to exercise remains incompletely understood.

Because exercise increases ventricular preload, afterload, contractility, and heart rate, which are the primary determinants of cardiac vagal afferent discharge, it is logical to predict that exercise should enhance cardiac baroreflex restraint on sympathetic outflow. Indeed, earlier studies in both animals and humans suggested that cardiac baroreceptor discharge attenuates reflex vasoconstriction during exercise. The concept advanced was that this attenuation is due to a central neural interaction between an excitatory input from thin fiber somatic afferents arising in the contracting muscles (i.e., “somatic or exercise pressor reflex”) and an inhibitory influence from vagal afferents arising from the cardiopulmonary region (i.e., “cardiac baroreflex”). More recently, however, the concept of this reflex interaction has been challenged by studies in conscious dogs in which circulatory responses to treadmill exercise were unaffected by vagal afferent section.

Accordingly, the goal of this study was to further test this concept with direct measurements of sympathetic nerve discharge using intraneural microelectrodes in conscious, exercising humans. We used static handgrip to activate muscle afferents and used nonhypotensive lower-body negative pressure (LBNP) to simulate orthostatic stress and unload cardiac afferents while measuring sympathetic outflow to nonexercising leg muscles as an index of the reflex response to these interventions. Handgrip and LBNP were performed alone and in combination to test the possibility of a reflex interaction. If the cardiac baroreflex exerts an inhibitory influence on sympathetic discharge during exercise, it follows that unloading of cardiac baroreceptors during LBNP should amplify sympathetic neural activation during supine static handgrip.

Subjects and Methods

Thirteen subjects (eight men and five women; age, 22–37 years) participated in this study after providing written informed consent. One subject was studied on two different occasions. All subjects were normotensive (supine blood pressures <140/90 mm Hg), were taking no medications, and had no evidence of cardiopulmonary disease on history or physical examination at the time of the study. The protocol was approved by the institutional review board on human investigation.

Procedures

All experiments were performed with the subjects supine and the lower body enclosed in a negative pressure chamber. A small door was created on the side of the LBNP chamber to allow performance of the microneurographic technique for recording muscle sympathetic nerve activity (MSNA) from the peroneal nerve in the right leg. Once a stable recording was obtained, the door was closed and sealed during the protocol. Heart rate (electrocardiogram), respiratory excursions (pneumograph), central venous pressure (intrathoracic catheter), dynamometer force (Stoelting handgrip dynamometer, Stoelting, Chicago, Illinois), and efferent MSNA (intraneural microelectrode) were recorded continuously on a Gould physiological recorder (model 280S, Gould, Statham, Oxnard, California) and on a Teac R-71 FM tape recorder (Teac, Japan). Respiration was monitored to detect inadvertent performance of a Valsalva maneuver or prolonged exhalation because these respiratory maneuvers markedly stimulate MSNA. No such maneuvers were detected during the experimental protocols. Blood pressure was measured in the right arm with sphygmomanometry. After subjects were given local anesthesia, a venous catheter (0.7 mm i.d., 60 cm length) was inserted percutaneously into an antecubital vein and advanced into an intrathoracic vein for measurement of central venous pressure, which was used as an index of the mechanical stimulus to the cardiac baroreceptors. Mean arterial pressure was calculated as diastolic pressure plus one third of the pulse pressure.

Microneurography

Multiunit recordings of postganglionic sympathetic nerve activity were obtained from muscle nerve fascicles in the right peroneal nerve posterior to the fibular head by microneurography. The details of this technique have been described previously. Briefly, unipolar recordings of MSNA were obtained with tungsten intraneural microelectrodes. The neural signals were amplified by a factor of 20,000–50,000-fold and filtered with a bandwidth of either 700–2,000 or 300–2,000 Hz. The filtered neurogram was rectified and integrated with a resistance-capacitance circuit (time constant, 0.1 seconds) to obtain a mean voltage display of MSNA. A recording of MSNA was considered acceptable when 1) electrical stimulation (1–3 V, 0.2 msec, 1 Hz) through the intraneural electrode produced muscle twitches but not paresthesias; 2) the receptive field of the impaled mecanoreceptor 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 neurogram revealed spontaneous, pulse-synchronous bursts that increased during prolonged exhalation and phases II and III of a Valsalva maneuver but not during arousal stimuli (loud noise, skin pinch). Neuro-
grams that revealed spontaneous skin sympathetic activity were not accepted. Sympathetic bursts were identified by inspection of the mean voltage neurogram. The neural activity was expressed both as bursts per minute (an index of the frequency of the activity) and as bursts per minute times mean burst amplitude (an index of integrated [total] activity). The intraobserver variability in identifying bursts is <5%, and the interobserver variability is <10%.20 Inadvertent contraction of the leg muscles adjacent to the recording electrode produces electromyographic artifacts that are easily distinguished from sympathetic bursts. Before beginning the protocol, subjects rested quietly for 10 minutes to ensure a stable level of baseline parameters. Resting nerve activity was recorded for 5 consecutive minutes.

Static Handgrip and Posthandgrip Muscle Ischemia

With the subject in the supine position, maximal voluntary contraction (MVC) was determined for the nondominant hand at the beginning of each experiment. Subjects were instructed to avoid performance of a Valsalva maneuver or a prolonged exhalation and to avoid contracting muscles other than those in the exercising forearm.

Subjects performed static handgrip at 20%, 25%, and 30% MVC for 2 minutes each to produce graded levels of muscle afferent stimulation and reflex increases in MSNA. In some experiments, forearm vascular occlusion (muscle ischemia) was produced by inflation of a pneumatic cuff on the upper exercising arm to suprasystolic pressure (250 mm Hg) 5 seconds before the end of the handgrip; after the cuff was inflated, the subjects were instructed to relax the grip while the circulatory arrest was maintained for an additional 2 minutes. The purpose for this procedure was to show that the exercise-induced increases in sympathetic activity in the resting leg were caused specifically by a reflex arising in the contracting arm muscles. Increases in sympathetic outflow during voluntary exercise have been attributed to impulses arising in the central nervous system (i.e., to central motor command)21-23 as well as to impulses arising in muscle afferents. Posthandgrip forearm vascular occlusion is a strategy to trap local metabolites in the vicinity of the muscle afferent endings and thus maintain the chemical stimulation of these afferents while the muscular relaxation eliminates central command.

Lower-Body Negative Pressure

Venous pooling was produced by application of negative pressure at −5 mm Hg to the lower body, which was enclosed in an airtight chamber to the level of the iliac crests. The pressure inside of the chamber was measured with a Statham pressure transducer. Because LBNP at −5 mm Hg has been shown to decrease central venous pressure but not to decrease mean arterial pressure, pulse pressure, or arterial dP/dt, the assumption was that this low level of LBNP primarily unloads the cardiac baroreceptors without producing an important alteration in the discharge of the sinoaortic baroreceptors.5,24

Protocols

Protocol 1: Handgrip during LBNP (36 experiments, nine subjects). Each subject performed four separate experimental sequences such that two levels of static handgrip were each performed with and without LBNP: 1) handgrip at 20% MVC alone, 2) handgrip at 30% MVC alone, 3) handgrip at 20% MVC during LBNP, and 4) handgrip at 30% MVC during LBNP. The order of the four interventions was randomized, with at least 10 minutes of rest between experimental sequences. Each sequence consisted of 2 minutes of 1) control, 2) handgrip, 3) posthandgrip muscle ischemia, and 4) recovery (relaxation without forearm vascular occlusion).

For the combined interventions (i.e., static handgrip during LBNP), a stable decrease in CVP was obtained for 2 minutes to achieve a new steady-state control level of MSNA upon which the handgrip–muscle ischemia sequence was superimposed. This level of LBNP (−5 mm Hg) was maintained until the end of the recovery period.

Protocol 2: LBNP during handgrip (26 experiments, five subjects). The purpose of these additional experiments was to change the order of the application of interventions such that the LBNP was performed during handgrip rather than handgrip during LBNP. Each subject performed three separate experimental sequences in a randomized order: 1) static handgrip at 25% MVC alone for 2 minutes, 2) LBNP at −5 mm Hg alone for 1 minute, and 3) LBNP during the 2nd minute of handgrip. In other words, with the combined intervention, subjects performed handgrip alone for the 1st minute, and then the LBNP was applied concomitantly during the 2nd minute of sustained handgrip. To test the possibility of a reflex interaction, we compared the algebraic sum of the individual responses to handgrip alone and to LBNP alone with the response to the combined intervention.

Protocol 3: Effects of filtering on quantitation of nerve traffic. In five subjects, we systematically varied the low-frequency filter on the nerve traffic analyzer to test effects of bandwidth on the quantitation of sympathetic activity. The raw, unfiltered neurogram was routed simultaneously through two separate filters, one with a bandwidth of 700–2,000 Hz (the standard setting) and the other with a bandwidth of 300–2,000 Hz. For comparative quantitative analysis, we produced simultaneous hard copy displays of the unfiltered signals, the filtered neurogram with the 700–2,000-Hz bandwidth and the corresponding mean voltage display, and the filtered neurogram with the 300–2,000-Hz bandwidth and that corresponding mean voltage display. Sympathetic bursts were analyzed by inspection of the mean voltage neurograms for 5 consecutive minutes of resting nerve activity. The neurograms
TABLE 1. Responses to Static Handgrip and to Posthandgrip Muscle Ischemia Without Lower-Body Negative Pressure

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Static handgrip</th>
<th>Posthandgrip muscle ischemia</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st minute</td>
<td>2nd minute</td>
<td>1st minute</td>
<td>2nd minute</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>95±2</td>
<td>92±3</td>
<td>94±3</td>
<td>102±3*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>62±3</td>
<td>62±2</td>
<td>68±3*</td>
<td>69±4*</td>
</tr>
<tr>
<td>Muscle sympathetic activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of activity (bursts/min)</td>
<td>14±2</td>
<td>12±2</td>
<td>11±2</td>
<td>24±3*</td>
</tr>
<tr>
<td>Total activity (bursts/min x mean burst amplitude)</td>
<td>209±33</td>
<td>187±25</td>
<td>167±41</td>
<td>422±56*</td>
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</table>

Handgrip at 30% MVC

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<th>Control period</th>
<th>Static handgrip</th>
<th>Posthandgrip muscle ischemia</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>94±2</td>
<td>93±3</td>
<td>97±3</td>
<td>109±3*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>59±3</td>
<td>60±3</td>
<td>74±4*</td>
<td>77±4*</td>
</tr>
<tr>
<td>Muscle sympathetic activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of activity (bursts/min)</td>
<td>12±2</td>
<td>9±2</td>
<td>14±2</td>
<td>35±2*</td>
</tr>
<tr>
<td>Total activity (bursts/min x mean burst amplitude)</td>
<td>188±36</td>
<td>154±32</td>
<td>204±30</td>
<td>689±80*</td>
</tr>
</tbody>
</table>

Entries are mean±SEM for nine subjects. Values for total muscle sympathetic nerve activity are expressed in arbitrary units. MVC, maximal voluntary contraction. *p<0.05, significantly different than average of control values.

Figure 1. Segments of an illustrative record from one subject showing both the filtered neurogram (FN) and the mean voltage neurogram (MVN) of muscle sympathetic activity (right peroneal nerve). Data represent the last 30 seconds of control, static handgrip (20% maximal voluntary contraction), posthandgrip forearm vascular occlusion, and recovery periods, respectively. Protocol was performed both without lower-body negative pressure (LBNP) (Panel A) and during LBNP at -5 mm Hg (Panel B). LBNP had no effect on the sympathetic nerve response to handgrip or to posthandgrip forearm vascular occlusion.
of each pair (300 vs. 700-Hz low-cut filter) were analyzed separately and blinded.

Protocol 4: Valsalva maneuver. The purpose of this protocol was to determine if the MSNA could exceed the maximal level achieved during the combined intervention. The last three subjects studied in Protocol 1 were asked to perform a strenuous Valsalva maneuver by exhaling into a mouthpiece connected to a manometer to maintain intrathoracic pressure > +40 mm Hg for at least 10 seconds. The response to phases II and III of the Valsalva maneuver was compared with that during the last 15 seconds of handgrip at 30% MVC during LBNP.

Data Analysis

Steady-state values of blood pressure, heart rate, central venous pressure, and MSNA were determined for each 60-second interval. Statistical analysis was performed by Statistical Analysis System software (SAS Institute, Cary, North Carolina) using analysis of variance with the Greenhouse-Geisser adjustment for lack of sphericity and the Bonferroni method for multiple comparisons.23 p < 0.05 was considered statistically significant. Results are expressed as the mean ± SEM.

Results

Protocol 1

Responses to static handgrip and to posthandgrip forearm ischemia without LBNP (Table 1 and Figures 1 and 2). As expected, handgrip alone caused significant (p < 0.05) and graded increases in arterial pressure and heart rate. During the 2nd minute of handgrip at 20% and 30% MVC, mean arterial pressure increased by +9 ± 3 and +16 ± 2 mm Hg over control values, while heart rate increased by +7 ± 2 and +16 ± 2 beats/min, respectively.

Static handgrip also caused graded increases in MSNA. During the 1st minute of handgrip, MSNA did not change significantly with either level of exercise (p > 0.1 vs. control), whereas, during the 2nd minute, MSNA increased significantly (p < 0.05 vs. control) by + 11 ± 3 bursts/min (+175 ± 89% increase in total activity) during handgrip at 20% MVC and by +24 ± 2 bursts/min (+431 ± 110% increase in total activity) during handgrip at 30% MVC.

During forearm vascular occlusion after handgrip, heart rate returned to the control level, whereas arterial pressure and MSNA remained significantly elevated (p < 0.05) above control. Blood pressure and MSNA returned to control during the recovery period (i.e., relaxation without vascular occlusion).

Responses to LBNP alone (Figures 1 and 2). LBNP alone at −5 mm Hg did not significantly alter mean arterial pressure (93 ± 2 mm Hg before LBNP vs. 92 ± 2 mm Hg during LBNP) or heart rate (62 ± 3 vs. 61 ± 2 beats/min) but decreased central venous pressure by 2.5 ± 0.1 mm Hg (p < 0.05) and increased MSNA from 10 ± 2 to 17 ± 2 bursts/min during LBNP (92 ± 22% increase in total activity, p < 0.05). MSNA increased progressively during the first 40 seconds of LBNP to reach a new steady-state level. This level of activity remained stable during the remaining 80 seconds of LBNP alone: four consecutive 20-second determinations of MSNA averaged

![Figure 2. Changes in muscle sympathetic nerve activity, mean arterial pressure, and heart rate that resulted from lower-body negative pressure (LBNP) at −5 mm Hg alone and from static handgrip (HG) at 20% and at 30% of maximal voluntary contraction performed alone (open bars) and during LBNP (hatched bars). Changes in each subject were calculated by subtracting the control value from the value obtained during the 2nd minute of the intervention. Note that LBNP increased the control level of neural activity upon which the handgrip responses were superimposed. LBNP did not affect the increases in muscle sympathetic activity, arterial pressure, or heart rate during these levels of handgrip. Data are mean±SEM for nine subjects.](http://circ.ahajournals.org/doi/abs/10.1161/01.RES.0000108947.07726.29)
272 ± 39, 260 ± 42, 249 ± 39, and 263 ± 55 units/min, respectively; none of these values differed significantly from the other (p > 0.1). Thus, LBNP caused a small but significant increase in the basal level of MSNA upon which the exercise stimulus was superimposed.

Responses to static handgrip and posthandgrip forearm ischemia during LBNP (Table 2 and Figures 1–4). Most important, handgrip at 20% and 30% MVC performed during LBNP produced increases in arterial pressure, heart rate, and MSNA that did not differ significantly from the increases produced by handgrip alone. In addition, the responses to posthandgrip forearm ischemia did not differ between the two experimental conditions.

Figures 3 and 4 demonstrate the time course of the MSNA response to static handgrip at 30% MVC performed alone and in combination with LBNP. Under both conditions, there was a 40–60-second lag in the onset of the MSNA response from the onset of contraction, and thereafter, MSNA increased steadily and comparably throughout the 2nd minute of handgrip. Thus, LBNP had no effect on either the time course or the magnitude of the MSNA response to static handgrip.

Protocol 2

LBNP during handgrip (Table 3). In this series of experiments, there were no differences in the control values of MSNA before the three interventions (LBNP alone, handgrip alone, and LBNP during handgrip). Both handgrip alone and LBNP alone caused significant (p < 0.05) increases in MSNA. Most important, concomitant application of handgrip plus LBNP caused increases in MSNA that were comparable with but not greater than the increases caused by handgrip alone (p > 0.1).

During LBNP alone, CVP decreased by 2.1 ± 0.3 mm Hg (p < 0.05 vs. control), whereas, during handgrip alone, CVP increased by 1.5 ± 0.3 mm Hg. During handgrip plus LBNP, CVP decreased by 0.6 ± 0.4 mm Hg below control; thus, CVP was 2.1 ± 0.5 mm Hg lower during the combined intervention than during handgrip alone (p < 0.05).

The increases in mean arterial pressure and heart rate caused by concomitant application of LBNP during handgrip were comparable with those caused by handgrip alone (+16 ± 2 vs. 20 ± 2 mm Hg, +20 ± 4 vs. +21 ± 5 beats/min, p > 0.1).

Protocol 3

Effects of filtering on quantitating nerve traffic (Figure 5). Resting values of MSNA in bursts per minute for 5 consecutive minutes with low-cut filters set at 300 and 700 Hz simultaneously were 24 ± 3 versus 26 ± 3, 24 ± 3 versus 23 ± 3, 24 ± 2 versus 24 ± 3, 23 ± 2 versus 22 ± 2, and 23 ± 2 versus 25 ± 2, respectively. Thus, increasing the low level of the bandpass filter from 300 to 700 Hz did not alter burst frequency.

Protocol 4

Comparison of peak MSNA responses during the handgrip and during the Valsalva maneuver (Table 4
and Figure 6). In each of the three subjects in whom this comparison was performed, the peak level of MSNA achieved during handgrip was substantially lower (47% lower on the average) than that during phases II and III of the Valsalva maneuver.

Discussion

Although cardiopulmonary baroreceptors with vagal afferents have been thought to exert an important inhibitory influence on sympathetic vasoconstrictor outflow during exercise, recent studies in conscious dogs by Walgenbach and Donald and Figure 3. Segments of an illustrative record from one subject showing effects of lower-body negative pressure (LBNP) on the time course of the muscle sympathetic nerve response to static handgrip at 30% of maximal voluntary contraction (MVC). Panels show the mean voltage neurogram (MVN) and the force tracing when handgrip was performed without LBNP (Panel A) and during LBNP (Panel B). For purposes of illustration, the record has been transcribed from FM tape at a paper speed of 1 mm/sec to demonstrate the entire handgrip sequence. Under both experimental conditions, the time course and the magnitude of the sympathetic nerve responses to static handgrip were comparable.

and Figure 6). In each of the three subjects in whom this comparison was performed, the peak level of MSNA achieved during handgrip was substantially lower (47% lower on the average) than that during phases II and III of the Valsalva maneuver.

Discussion

Although cardiopulmonary baroreceptors with vagal afferents have been thought to exert an important inhibitory influence on sympathetic vasoconstrictor outflow during exercise, recent studies in conscious dogs by Walgenbach and Donald did not demonstrate an effect of these vagal afferents on blood pressure and peripheral vasoconstrictor responses to exercise. Therefore, to reexamine the role played by the cardiac baroreflex in modulating the reflex sympathetic response to exercise in humans, we tested effects of reduction in cardiac filling pressure induced by LBNP on sympathetic nerve discharge to non-exercising muscle during sustained handgrip. The principal new conclusion is that unloading of the cardiac baroreflex with nonhypotensive LBNP does not alter the muscle sympathetic nerve response to static handgrip either in time course or in magnitude. Our observations support and extend the above findings in exercising dogs and challenge the concept of an important interaction between cardiac baroreceptor and somatic pressor reflexes during static exercise in humans.

This interpretation of the sympathetic nerve responses is contingent on: 1) the evidence that increases in MSNA during handgrip represent a specific reflex response to muscle contraction, 2)

![Figure 3](image3.png)

**Figure 3.** Segments of an illustrative record from one subject showing effects of lower-body negative pressure (LBNP) on the time course of the muscle sympathetic nerve response to static handgrip at 30% of maximal voluntary contraction (MVC). Panels show the mean voltage neurogram (MVN) and the force tracing when handgrip was performed without LBNP (Panel A) and during LBNP (Panel B). For purposes of illustration, the record has been transcribed from FM tape at a paper speed of 1 mm/sec to demonstrate the entire handgrip sequence. Under both experimental conditions, the time course and the magnitude of the sympathetic nerve responses to static handgrip were comparable.

![Figure 4](image4.png)

**Figure 4.** Total muscle sympathetic nerve activity (expressed as a percentage of the control value) plotted for each 20-second interval of static handgrip at 30% of maximal performed both without lower-body negative pressure (LBNP) (—), and during LBNP at −5 mm Hg (○). Data are mean±SEM for nine subjects. *p<0.05, significantly different from control. Under both conditions, the sympathetic activity began to increase only toward the end of the 1st minute of handgrip and then increased steadily throughout the 2nd minute. LBNP neither shortened the time to the onset of sympathoexcitation in the 1st minute of handgrip nor augmented the increases in nerve traffic in the 2nd minute.

![Table 3](image3.png)

**Table 3.** Responses to Lower-Body Negative Pressure Alone, to Static Handgrip Alone, and to Lower-Body Negative Pressure Applied During Handgrip (Protocol 2)

<table>
<thead>
<tr>
<th></th>
<th>Frequency of activity (bursts/min)</th>
<th>Total activity (bursts/min × mean burst amplitude)</th>
<th>Δ</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Control</td>
<td>Intervention</td>
<td>Δ</td>
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<tr>
<td>LBNP alone</td>
<td>26 ± 3</td>
<td>33 ± 3*</td>
<td>+7 ± 2</td>
</tr>
<tr>
<td>Handgrip at 25% MVC alone</td>
<td>26 ± 2</td>
<td>39 ± 6*</td>
<td>+13 ± 4</td>
</tr>
<tr>
<td>Handgrip at 25% MVC plus LBNP</td>
<td>24 ± 2</td>
<td>46 ± 4*</td>
<td>+22 ± 2</td>
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</tbody>
</table>

Entries are mean±SEM for five subjects. Values for total muscle sympathetic nerve activity are expressed in arbitrary units. LBNP, lower-body negative pressure; MVC, maximal voluntary contraction.

*p<0.05, significantly different from control.
the ability of LBNP at only $-5$ mm Hg to effectively and selectively unload cardiac baroreceptors, 3) the possible effects of the initial level of nerve activity on the responses to handgrip, 4) the possibility that signal processing caused loss of neural information, 5) the possibility that handgrip alone produced maximal increases in MSNA (i.e., a ceiling effect), and 6) the potentially confounding effects of arterial baroreflexes.

There is now persuasive evidence that the increases in peroneal MSNA during static handgrip are directly related to the forearm exercise. First, the magnitude of this response is graded to the intensity of the muscle contraction. Second, the response is characterized by a highly consistent and reproducible pattern of sympathetic activation. Third, the primary mechanism of this sympathetic nerve response appears to be the stimulation of chemically sensitive muscle afferents in the exercising arm.\textsuperscript{20,26} In the present as well as in previous experiments,\textsuperscript{20} when handgrip is followed by posthandgrip forearm vascular occlusion to prevent washout of local metabolites from the vicinity of the muscle afferent endings, the sympathoexcitatory response in the leg is maintained even though the muscular relaxation eliminates other factors such as mechanically sensitive muscle afferents and the volitional component of exercise, termed central command.\textsuperscript{21-23} The finding that LBNP did not augment the MSNA responses to either handgrip exercise (i.e., muscle afferents plus central command) or posthandgrip forearm vascular occlusion (i.e., chemically sensitive muscle afferents alone) suggests that our inability to demonstrate an interaction of these somatic and cardiac reflexes was not due to a confounding influence of central command.

The finding that LBNP alone at $-5$ mm Hg produced sustained decreases in CVP and increases in MSNA indicates that even this low level of negative pressure effectively unloaded cardiac baroreceptors under resting conditions. However, because static exercise caused substantial increases in arterial pressure, we monitored CVP continuously to determine if the increases in arterial pressure and ventricular afterload might have impaired the ability of LBNP to decrease cardiac filling pressure during the handgrip. This was not the case; although static handgrip alone indeed caused increases in CVP, LBNP decreased CVP as much during handgrip as it did under nonexercising conditions (i.e., by $-2$ mm Hg). Taken together, these observations suggest that this low level of negative pressure was an effective intervention to decrease cardiac baroreflex activity and to study the postulated reflex interaction. Furthermore, because the sympathoexcitatory response to LBNP alone was not accompanied by decreases in arterial pressure or increases in heart rate, the present findings are consistent with previous observations that strongly suggest that the primary mechanism of this sympathetic nerve response is unloading of cardiac rather than arterial baroreceptors.\textsuperscript{5,7,9,24} While we obviously cannot exclude a minor role for the arterial baroreflex in these human experiments, the finding that the increases in MSNA during LBNP were not accompanied by corresponding increases in heart rate argues against an important perturbation in arterial baroreceptor activity.\textsuperscript{19}

In our initial experiments, we applied the suction before the onset of exercise to examine effects of cardiac baroreceptor unloading on the time course of MSNA response to handgrip and to simulate the clinical condition of exercise performed after assumption of upright posture. Although one interpretation of the results is that the cardiac baroreflex does not modulate the MSNA response to static handgrip, a potentially confounding factor in this experimental design was that the preexercise level of sympathetic nerve activity was almost 100% higher than normal when handgrip was preceded by LBNP. However, this factor does not appear to account for the failure of LBNP to augment the MSNA response to handgrip because we also found no evidence for a reflex

<table>
<thead>
<tr>
<th>Subject</th>
<th>Static handgrip at 30% MVC during LBNP</th>
<th>Valsalva maneuver (phases II and III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>768</td>
<td>954</td>
</tr>
<tr>
<td>2</td>
<td>768</td>
<td>1,948</td>
</tr>
<tr>
<td>3</td>
<td>568</td>
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<tr>
<td>Mean ± SEM</td>
<td>701 ± 66</td>
<td>1,325 ± 313</td>
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</table>

Values of total muscle sympathetic nerve activity were calculated during the last 15 seconds of handgrip and during phases II and III of the Valsalva maneuver. Activity is expressed in units per minute (bursts/min × mean burst amplitude).

MVC, myocardial voluntary contraction; LBNP, lower-body negative pressure.
interaction in a subsequent series of experiments in which we began the LBNP after rather than before the onset of the handgrip; under these conditions, the control levels of nerve activity before handgrip alone and before handgrip plus LBNP were practically identical.

While it is therefore unlikely that we overlooked an important cardiac-somatic reflex interaction, we considered the possibility that a subtle interaction might have been obscured because of loss of neural information caused by signal processing. The band-pass filter used to analyze nerve traffic was substantially narrower in these human experiments than that routinely used in animal experiments; animals with heart rates comparable to humans have significant nerve activity at frequencies lower than 700 Hz. We suggest that this processing did not affect the interpretation of our results. The amount of detectable sympathetic activity did not increase when we decreased the low-cut filter from 700 to 300 Hz.

We considered the important possibility that static handgrip alone might have produced near-maximal increases in MSNA and thus precluded an augmentation of this response by LBNP. Several lines of evidence indicate that this was not the case. First, the sympathoexcitatory response to handgrip at 20% and 30% MVC was clearly graded, which demonstrates that, with the lower level of exercise, the increases in MSNA were not maximal. Second, with the higher level of exercise, the MSNA rose steadily throughout the entire 2nd minute of handgrip without showing any evidence of reaching a plateau. Third, and most important, three of the subjects performed a strenuous Valsalva maneuver, which, in each subject, resulted in a much larger increase in MSNA than the peak increase observed during the handgrip. Taken together, these observations strongly suggest that the intensity-dependent responses to handgrip fell on the steep portion of the stimulus-response curve relating somatic reflex activation to efferent sympathetic discharge.

A final consideration relates to the influence of sinoaortic baroreceptors. The exercise-induced increases in arterial pressure would be expected to stimulate arterial baroreceptor discharge that might have overridden the sympathoexcitatory effect of cardiac baroreceptor unloading by LBNP. However, Walgenbach and Donald and Daskalopoulos et al were unable to show that vagal afferents modulate the pressor and peripheral vasoconstrictor responses to exercise in dogs even after arterial baroreceptor input was removed by sinoaortic denervation.

Although the effects of exercise on arterial baroreflex function are incompletely understood, the current thinking is that exercise causes the arterial baroreceptors to operate at a set point that is higher than normal. This resetting might attenuate arterial baroreflex buffering of the MSNA response to static exercise. In this regard, two observations strongly suggest that arterial baroreceptors did not preclude the demonstration of an interaction between cardiac and somatic reflex control of muscle sympathetic outflow. First, the finding that MSNA increased markedly during handgrip alone in spite of substantial increases in arterial pressure demonstrates that arterial baroreflex buffering does not prevent the sympathoexcitatory response to static exercise. Second, because the arterial pressure responses to handgrip alone and to handgrip plus LBNP were of comparable magnitude, it is difficult to explain the lack of a difference in the sympathetic nerve responses to these two interventions on the basis of arterial baroreceptors.

Our conclusions based on intraneural recordings of muscle sympathetic activity are different than those of Thames and Abboud and Walker et al, which are based on measurements of regional blood flow and vascular resistance. The concept that cardiac baroreceptors modulate reflex vasoconstrictor responses to somatic afferent stimulation was based on the following observations: 1) in dogs, volume expansion attenuated and vagotomy augmented the renal vasoconstrictor responses to sciatic nerve stimulation; and 2) in humans, LBNP at −5 mm Hg augmented the forearm vasoconstrictor responses to static handgrip at both 10% and 20% MVC.

Although the effector responses to muscle sympathetic nerve discharge in humans are incompletely understood, there is compelling evidence that this neural activity principally results in noradrenergic vasoconstriction. Therefore, it is diffi-
cult to explain why nonhypotensive LBNP potenti-
ates vasoconstriction in the resting forearm but
does not potentiate sympathetic activation in the
resting calf. A possible explanation might relate to
differential effects of cardiac baroreflex activity on
sympathetic vasomotor responses in the arm as
compared with the leg. Indeed, there is increasing
evidence that many reflex sympathetic stimuli pro-
duce differential vascular responses in the forearm
and calf,32 and, more specifically, Essandoh et
al33,34 have recently suggested that unloading of
cardiac receptors with nonhypotensive LBNP causes
vasoconstriction in the forearm but not in the calf.
While one could, therefore, postulate that the car-
diac baroreflex preferentially augments somatic
reflex responses in the arm and not in the leg, it is
unlikely that, in our subjects, LBNP produced
widespread augmentation of sympathetic activation
in tissues and vascular beds other than the leg
muscles because the exercise-induced increases in
blood pressure and heart rate were not augmented by
LBNP.

In summary, our findings do not support the
notion of a major interplay between reflexes arising
in the heart and reflexes arising in exercising skel-
etal muscles in the control of muscle sympathetic
outflow in humans. Based on our experimental
findings in healthy subjects, we speculate that, in
the clinical setting, differences in cardiac barorecep-
tory activity between upright and supine positions
should have little effect on sympathetic neural con-
trol of the circulation during exercise testing in
patients.

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