Relative Contributions of Cardiopulmonary and Sinoaortic Baroreflexes in Causing Sympathetic Activation in the Human Skeletal Muscle Circulation During Orthostatic Stress

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The aim of this study was to reexamine the hypothesis that cardiopulmonary baroreflexes are more important than sinoaortic baroreflexes in causing vasoconstriction in the skeletal muscle circulation during orthostatic stress. We recorded muscle sympathetic nerve activity (MSNA) with microelectrodes in the peroneal nerve (and forearm blood flow with venous occlusion plethysmography) in normal subjects (innervated ventricles) and in heart transplant recipients (denervated ventricles) during graded lower body negative pressure (LBNP) performed alone and in combination with intravenous infusion of phenylephrine, which was titrated to eliminate the orthostatically induced fall in blood pressure and thus the unloading of both carotid and aortic baroreceptors. The principal new findings are as follows: (1) The increases in both MSNA and forearm vascular resistance during multiple levels of LBNP were not attenuated by heart transplantation, which causes ventricular but not sinoaortic deafferentation. (2) In heart transplant recipients, a small increase in MSNA during mild LBNP was dependent on a decrease in arterial pressure, but in normal subjects, a similar increase in MSNA occurred in the absence of any detectable decrease in the aortic pressure stimulus to the sinoaortic baroreceptors. (3) In normal subjects, the large increase in MSNA during a high level of LBNP was dependent on a decrease in arterial pressure and could be dissociated from the decrease in central venous pressure. Taken together, the findings strongly suggest that sinoaortic baroreflexes are much more important and ventricular baroreflexes are much less important than previously thought in causing reflex sympathetic activation and vasoconstriction in the human skeletal muscle circulation during orthostatic stress. (Circulation Research 1993;73:367-378)

KEY WORDS • sympathetic nervous system • ventricular sensory receptors • baroreceptor reflexes

Ventricular mechanoreceptors with C-fiber vagal afferents have been firmly established to reflexly inhibit sympathetic vasomotor outflow in experimental animals,1 but the role played by these afferents in the reflex control of the human cardiovascular system remains incompletely understood.

Previous hemodynamic studies in humans have suggested that cardiac filling pressure and contractility, the primary determinants of ventricular mechanoreceptor C-fiber discharge in experimental animals,2,3 also are primary determinants of efferent sympathetic vasomotor activation during orthostatic stress.4-6 During simulated orthostatic stress with lower body negative pressure (LBNP), β-adrenergic blockade, which decreases ventricular contractility in normal humans, and heart transplantation, which produces ventricular deafferentation, each were found to greatly attenuate the increase in forearm vascular resistance induced by a given decrease in central venous pressure.4-6 In contrast, prevention of carotid baroreceptor deactivation during LBNP was found to have no effect on this vasoconstrictor response, supporting the theory that, during orthostatic stress, unloading of cardiopulmonary, not sinoaortic, baroreceptors is the primary mechanism causing reflex vasoconstriction in the peripheral circulation.7,8 However, recent data from intraneural recordings of muscle sympathetic nerve activity (MSNA) call this theory into question. In normal humans, decreases in ventricular contractility with β-adrenergic blockade had no effect on the reflex increases in MSNA evoked by decreases in central venous pressure,9 negating one of the principal pieces of evidence linking reflex vasoconstriction to ventricular mechanoreceptor deactivation. The aim of this study therefore was to reexamine the remaining experimental evidence that ventricular mech-
anoreceptor deactivation triggers the reflex vasoconstriction in the peripheral circulation during orthostatic stress. Specifically, we asked whether the reflex increases in sympathetic vasoconstrictor outflow to the skeletal muscle bed produced by LBNP are impaired, or not impaired, either by (1) heart transplantation, which causes ventricular but not sinoaortic deafferentation, or (2) pharmacological elimination of the orthostatically induced fall in arterial but not central venous pressure, thereby preventing the deactivation of sinoaortic but not ventricular (or other cardiopulmonary) baroreceptors. To address these questions, we recorded MSNA with microelectrodes in the peroneal nerve in normal subjects (innervated ventricles) and in heart transplant recipients (denervated ventricles) during graded LBNP performed alone and in combination with a nonhypertensive infusion of phenylephrine.

**Subjects and Methods**

The protocols were approved by the institutional review board for human investigation, and all subjects provided informed written consent.

We studied a total of 73 subjects, including 34 heart transplant recipients and 39 age-matched normal control subjects. Subjects consisted of (1) 34 heart transplant recipients (31 men and 3 women, 51±3 years of age) who had undergone orthotopic cardiac transplantation either at the University of Wisconsin, Madison, or at the University of Texas Southwestern Medical Center, Dallas, within the past 1 to 46 months and (2) 39 control subjects (36 men and 3 women, 47±3 years of age). Thirty-six of the 39 control subjects were normotensive (blood pressure, <140/90 mm Hg), whereas 3 of the control subjects had mild essential hypertension (blood pressure, <170/100 mm Hg). None of the control subjects had evidence of active cardiopulmonary disease by history or physical examination.

At the time of study, none of the transplant recipients had evidence of graft rejection or congestive heart failure as assessed by history, physical examination, electrocardiograms, rest and exercise multigated acquisition scans, echocardiograms, and right and left heart catheterization with endomyocardial biopsy. All transplant recipients were outpatients and were physically active (enrolled in a cardiac rehabilitation program) at the time of study. The immunosuppressive regimens of all patients included cyclosporine (mean dose, 4.2±0.2 mg/kg per day), azathioprine (122±11 mg per day), and prednisone (16±2 mg per day).

Nineteen of the 34 transplant recipients and 3 of the 39 control subjects were taking antihypertensive medications before entry into the study. All antihypertensive medications were withheld for at least 72 hours before study.

**General Procedures**

Arterial pressure was measured by sphygmomanometry in the right arm using a Dinamap automated system (Critikon, Tampa, Fla). In a few experiments, arterial pressure was also measured by Finapress (Ohmeda, Englewood, Colo) for illustrative purposes. In a few experiments, central aortic pressure and its first derivative were measured using a high-fidelity catheter (Millar, Houston, Tex) with an analog differentiator (Gould, Cleveland, Ohio). Respiratory excursions were monitored with a strain-gauge pneumograph; during the experimental protocols, subjects were instructed to avoid performance of a Valsalva maneuver or prolonged expiration because these respiratory maneuvers can stimulate MSNA.

Heart rate was measured from the continuous electrocardiogram. In the heart transplant recipients, modified Lewis leads were used to detect the P waves of both the denervated donor atrium and innervated recipient atrial remnant. Changes in the rates of the innervated atrial remnants were used as an index of baroreflex control of sinus node function.

Heart rates, respiratory excursions, and MSNA were recorded continuously on an R-71 FM tape recorder (TEAC, Japan) and later transcribed to hard copy using an electrostatic recorder (model ES1000, Gould, Cleveland, Ohio).

**Recording of Sympathetic Nerve Discharge**

Multiunit recordings of postganglionic sympathetic nerve activity were obtained with unipolar tungsten microelectrodes inserted selectively into muscle nerve fascicles of the peroneal nerve posterior to the fibular head by microneurography. Briefly, the neural signals were amplified (by 20x10^5 to 50x10^5), filtered (bandwidth, 700 to 2000 Hz), rectified, and integrated (time constant, 0.1 second) to obtain a mean voltage display of sympathetic activity. A recording of muscle sympathetic activity was considered acceptable when the neurograms revealed spontaneous pulse-synchronous bursts of neural activity, with a minimum signal-to-noise ratio of 2:1, that increased during phases II and III of the Valsalva maneuver but not during arousal stimuli (loud noise, skin pinch).

Sympathetic bursts were detected by inspection of the filtered and mean voltage neurograms; the interobserver and intraobserver variability in identifying bursts was <10% and <5%, respectively. Inadvertent contraction of the leg muscles adjacent to the recording electrode produces electromyographic artifacts that are easily distinguished from sympathetic bursts; neurograms that revealed such artifacts were excluded from analysis. Nerve traffic was expressed as (1) the number of bursts of sympathetic activity per minute and per 100 heart beats, indexes of frequency of activity; (2) mean burst amplitude, which reflects the average amount of sympathetic activity contained within each burst; and (3) the number of bursts per minute times mean burst amplitude, an index of integrated nerve activity. For quantitative analysis, neurograms were transcribed from FM tape to hard copy such that baseline noise levels and mean burst amplitudes were roughly comparable in heart transplant recipients and control subjects.

**Measurement of Forearm Blood Flow**

Forearm blood flow was measured with venous occlusion plethysmography using air-filled latex cuffs. The forearm was elevated above the level of the right atrium to collapse the forearm veins. The circulation to the hand was arrested during blood flow measurements, which were performed at 15-second intervals. Forearm vascular resistance (in units) was calculated as mean arterial pressure (one-third pulse pressure plus diastolic pressure) divided by forearm blood flow in milliliters per 100 mL tissue per minute.
Lower Body Negative Pressure

The subject’s lower body was enclosed in a negative pressure chamber to the level of the iliac crest. An opening was created on one side of the chamber to allow performance of the microneurographic technique for recording sympathetic activity from the peroneal nerve in the right leg. Once a stable recording of sympathetic activity was obtained, the opening was closed and sealed during the protocol. The pressure inside of the LBNP chamber was measured by a Statham transducer (Gould, Oxnard, Calif).

Echocardiographic Measurements of Left Ventricular Dimensions

Left ventricular end-diastolic dimension was measured by two-dimensional echocardiography using a parasternal short-axis view according to the criteria of the American Society of Echocardiography. Decreases in left ventricular end-diastolic dimension were used as an index of the degree of orthostatic stress induced by graded LBNP.

Experimental Protocols

Protocol 1: Comparative MSNA responses to LBNP at −5, −10, and −15 mm Hg in heart transplant recipients (n=12) vs normal subjects (n=11). In this initial series of experiments, the aim was to test the hypothesis that ventricular baroreflexes are more important than sinoaortic baroreflexes in causing increases in MSNA during simulated orthostatic stress. We used low levels of LBNP, which in normal subjects are thought to unload mainly cardiopulmonary baroreceptors, not sinoaortic baroreceptors. Echocardiographic measurements of left ventricular end-diastolic dimension were used as an index of the stimulus to the ventricular baroreceptors. After a stable recording of MSNA had been obtained, subjects rested quietly for 30 minutes at the beginning of the experimental protocol, and then graded LBNP was applied at −5, −10, and −15 mm Hg for 5 minutes at each consecutive level. In a subset of five transplant recipients, we repeated these experiments after rapid intravenous infusion of 500 mL normal saline to exclude hypovolemia.

Protocol 2: Effects of intravenous phenylephrine on responses to LBNP at −15 mm Hg in heart transplant recipients (n=9). The results of protocol 1 indicated that arterial pulse pressure narrowed abnormally during mild (normal nonhypotensive) levels of LBNP in heart transplant recipients. Accordingly, the aim of this protocol was to determine if an increase in MSNA during a low level of LBNP in heart transplant recipients is dependent on this fall in blood pressure. Therefore, we restudied 9 of the initial 12 patients, in which LBNP at −15 mm Hg was repeated for 10 minutes and then the LBNP was continued while phenylephrine was infused intravenously with the dose (0.5 to 2.0 μg/kg per minute) being titrated to restore arterial pulse pressure to the baseline value.

Protocol 3: Comparison of forearm vascular responses during LBNP at −40 mm Hg in heart transplant recipients (n=10) vs normal control subjects (n=10). Because protocol 1 showed that the MSNA response to LBNP was not attenuated after heart transplantation, we performed this protocol to determine if we could replicate the earlier observation of an attenuated forearm vasoconstrictor response. In this protocol, we used LBNP at −40 mm Hg, since this was the highest level of LBNP used in the previous studies and showed the largest difference between heart transplant recipients and normal subjects. Forearm blood flow, heart rate, and blood pressure (sphygmomanometry) were measured at baseline and during application of LBNP at −40 mm Hg for 5 minutes. In this protocol, blood pressure was also measured continuously by Finapres so that we could display a hard-copy record of blood pressure. All patients were studied 6 to 24 months after transplantation.

Protocol 4: Relation between forearm vasoconstriction during LBNP at −40 mm Hg and time elapsed after heart transplantation. The results from protocol 3 did not replicate the earlier reports, indicating an attenuated forearm vasoconstrictor response to LBNP in heart transplant recipients. The aim of this protocol was to determine if the discrepancy in vascular findings might be related in part to the time that the patients were studied after heart transplantation, which was considerably shorter in the previous studies than in ours (1 to 12 vs 6 to 24 months). Forearm blood flow and blood pressure (sphygmomanometry) were measured at baseline and during LBNP at −40 mm Hg for 5 minutes in four more patients studied 2 to 3 months after heart transplantation; two of these subjects were restudied 6 months after transplantation. The data from these patients were compared with those from the patients in protocol 3.

Protocol 5: Comparison of arterial pressure and heart rate responses during assumption of upright posture in heart transplant recipients (n=8) vs normal subjects (n=9). The aim of this protocol was to examine the regulation of arterial pressure and heart rate during actual, rather than simulated, orthostatic stress. After resting quietly in the supine position for 30 minutes, subjects were instructed to assume the upright posture and to stand quietly for 60 seconds while arterial pressure (sphygmomanometry) and donor and recipient heart rates (electrocardiography) were measured.

Protocol 6: Comparison of sinoaortic baroreflex function in heart transplant recipients (n=7) vs normal subjects (n=7) during pharmacological alterations in arterial pressure. The aim of this protocol was to determine if the sympathetic nerve, as well as the heart rate, component of the sinoaortic baroreflex is normal, attenuated, or augmented in heart transplant recipients. After obtaining stable baseline recordings, arterial pressure, heart rate, and MSNA were recorded during both nitroprusside-induced decreases and phenylephrine-induced increases in arterial pressure. The nitroprusside infusion was titrated to produce graded reductions in mean arterial pressure of approximately −5, −10, and −15 mm Hg (dose range, 0.5 to 4.0 μg/kg per minute). The phenylephrine infusion was titrated to produce graded elevations in mean arterial pressure of approximately +5, +10, and +15 mm Hg (dose range, 0.5 to 2.5 μg/kg per minute).

Because the precise graded changes in mean arterial pressure were necessarily slightly different between the groups of subjects, we could not perform an unpaired comparison between individual points on the baroreflex curves. Therefore, we calculated the slopes of these lines for each subject and performed an unpaired t test between the groups.
Protocol 7: Effects of intravenous phenylephrine on MSNA responses to LBNP at -40 mm Hg in normal subjects (n=9). Based on the results suggesting the importance of sinoaortic baroreflexes in increasing MSNA during orthostatic stress in heart transplant recipients, this protocol was designed to reevaluate the relative contributions of sinoaortic vs cardiopulmonary baroreflexes in the activation of MSNA during hypotensive orthostatic stress in normal humans. In nine normal subjects, we measured blood pressure (sphygmonanometer), central venous pressure, and MSNA during LBNP at -40 mm Hg alone and in combination with intravenous infusion of phenylephrine (0.5 to 2.0 μg/kg per minute) titrated to restore arterial blood pressure to the baseline value and thereby offset the unloading of the sinoaortic baroreceptors. Because in some experiments central venous pressure during LBNP at -40 mm Hg increased slightly during the infusion of phenylephrine, the LBNP was increased to approximately -45 mm Hg so that central venous pressure would be decreased to the same amount during LBNP plus phenylephrine as during LBNP at -40 mm Hg alone.

Protocol 8: Effects of graded LBNP on central aortic blood pressure and dP/dt in normal subjects (n=4). The aim of this protocol was to determine if low levels of LBNP, which have been assumed to be nonhypotensive,16 in fact cause subtle decreases in arterial pressure that could be responsible for the LBNP-induced increases in MSNA. In eight experiments performed on four of our normal control subjects (previously studied in protocol 7), we measured central aortic pressure with a high-fidelity 2F Millar pressure transducer during graded LBNP at -5, -10, -15, -20, and -40 mm Hg (2 minutes at each successive level). Under local anesthesia with 1% lidocaine, the catheter was inserted percutaneously into the brachial artery, and under fluoroscopic guidance, the tip of the catheter was positioned in the aortic arch, i.e., in the proximity of the aortic arch baroreceptors. To obtain measurements of dP/dt, the pressure signal from the high-fidelity catheter was processed through an analog differentiator.

Data Analysis

Data were analyzed using repeated-measures analysis of variance with Dunnett’s post hoc tests to detect values that were statistically different from baseline values. Unpaired t tests were used to compare baseline values between groups and to compare group differences in baroreflex slopes. Values of P of <.05 were considered statistically significant. Data are expressed as mean±SEM.

Results

Baseline values of arterial pressure, ventricular heart rates, and MSNA all were significantly higher (P<.05) in the heart transplant recipients than in the normal control subjects (Tables 1, 3, and 4). In contrast, the
rates of innervated atrial remnants in the patients were comparable to the sinus rates in the normal subjects.

**Protocol 1: Comparative MSNA Responses to LBNP at −5, −10, and −15 mm Hg in Heart Transplant Recipients vs Normal Subjects**

In normal subjects, these mild levels of LBNP, as expected, caused significant (P<.05 vs baseline) and graded increases in MSNA without decreasing arterial pressure or increasing heart rate (Table 1 and Fig 1). The LBNP also caused significant increases in MSNA in heart transplant recipients, but these increases in MSNA were accompanied by significant (P<.05 vs baseline) decreases in arterial pulse pressure and increases in remnant atrial heart rates. LBNP at −15 mm Hg caused comparable decreases in left ventricular end-diastolic dimension (5.1±0.1 to 4.8±0.1 vs 5.1±0.1 to 4.9±0.1 cm) and approximately comparable increases in MSNA in transplant recipients and normal subjects. In a subset of five transplant recipients, decreases in arterial pulse pressure and increases in MSNA during LBNP at −15 mm Hg were unaffected by pretreatment with intravenous infusion of 500 mL normal saline (pulse pressure, −10±1 vs −9±1 mm Hg; change in integrated MSNA, +697±129 vs +668±99 units, P>.10).

Essential hypertension was present in 3 of the 11 control subjects. The increases in the integrated MSNA during LBNP at −15 mm Hg in these subjects were indistinguishable from those in the 8 normotensive control subjects: +303±50 vs +289±44 units (P>.10).

**Protocol 2: Effects of Intravenous Phenylephrine on Responses to LBNP at −15 mm Hg in Heart Transplant Recipients**

During LBNP at −15 mm Hg, increases in MSNA and in remnant atrial heart rates were almost completely abolished (P<.05 vs responses to LBNP alone) by restoring pulse pressure to normal with a concomitant infusion of phenylephrine (Table 2 and Fig 2).

**Protocol 3: Comparison of Forearm Vascular Responses During LBNP at −40 mm Hg in Heart Transplant Recipients vs Normal Control Subjects**

LBNP at −40 mm Hg produced increases in forearm vascular resistance in heart transplant recipients that were indistinguishable from those seen in normal control subjects. Mean arterial pressure decreased during LBNP at −40 mm Hg in both groups but to a greater
extent \((P<.05)\) in the heart transplant recipients (Table 3 and Fig 3).

**Protocol 4: Relation Between Forearm Vasoconstriction During LBNP at \(-40\) mm Hg and Time Elapsed After Heart Transplantation**

LBNP at \(-40\) mm Hg caused much smaller increases in forearm vascular resistance and arterial pressure when patients were studied within the first 2 to 3 months after heart transplantation than when studied more than 6 months after transplantation (Fig 4). In the two patients who were studied twice, forearm vascular resistance increased by only 4 and 6 units at 2 months but by 23 and 13 units, respectively, when restudied at 6 months after transplantation. Two to 3 months after transplantation, this high level of LBNP induced only a small nonsignificant fall in mean arterial pressure, from 111±6 to 108±6 mm Hg \((P>10)\).

**Protocol 5: Comparison of Arterial Pressure and Heart Rate Responses During Assumption of Upright Posture in Heart Transplant Recipients vs Normal Subjects**

During assumption of fully upright posture, mean arterial pressure fell by only 5±2 mm Hg in the normal subjects but by 17±1 mm Hg in the heart transplant recipients \((P<.05)\) (Table 4). In the patients, this exaggerated postural fall in arterial pressure was accompanied by increases in remnant atrial heart rates that were almost two times greater than the increases in sinus rates in the normal subjects \((P<.05)\). In contrast, the donor (ie, ventricular) heart rates did not change during postural adjustment.

**Protocol 6: Comparison of Sinoaortic Baroreflex Function in Heart Transplant Recipients vs Normal Subjects During Pharmacological Alterations in Arterial Pressure**

During infusions of nitroprusside and phenylephrine, alterations in arterial pressure had almost no effect on donor (ie, ventricular) heart rates but caused changes in innervated recipient remnant atrial heart rates that were comparable to the increases in sinus rates in the normal subjects (Fig 5). Phenylephrine-induced increases in arterial pressure evoked comparable decreases in MSNA in patients and normal subjects. However, nitroprusside-induced decreases in arterial pressure evoked threefold larger increases in mean burst amplitude in transplant recipients vs normal subjects \((P<.05)\) but comparable increases in integrated MSNA.

**Protocol 7: Effects of Intravenous Phenylephrine on MSNA Responses to LBNP at \(-40\) mm Hg in Normal Subjects**

Graded LBNP from \(-10\) to \(-40\) mm Hg caused expected decreases in central venous pressure and arterial pressure and increases in heart rate and MSNA (Table 5 and Fig 6). When we offset the fall in arterial pressure during LBNP at \(-40\) mm Hg with a concomitant infusion of phenylephrine but maintained the large fall in central venous pressure by increasing the LBNP slightly (from \(-40\) to approximately \(-45\) mm Hg), heart rate returned to baseline, and MSNA returned to a value that was equivalent to that produced by LBNP at \(-10\) to \(-15\) mm Hg alone.

**Protocol 8: Effects of Graded LBNP on Central Aortic Blood Pressure and dP/dt in Normal Subjects**

LBNP from \(-5\) to \(-15\) mm Hg had no detectable effects on systolic, diastolic, mean arterial, or pulse
pressures or on aortic dP/dt as measured with a high-fidelity Millar catheter in the aortic arch (Table 6 and Fig 7). LBNP from −20 to −40 mm Hg caused statistically significant and progressive decreases in arterial pressure and aortic dP/dt.

Discussion

The principal new conclusions from this study are as follows: (1) The increases in both MSNA and forearm vascular resistance during multiple levels of LBNP are not attenuated by heart transplantation, which causes ventricular but not sinoaortic deafferentation. (2) In heart transplant recipients, a small increase in MSNA during mild LBNP is dependent on a decrease in arterial pressure, but in normal subjects, a similar increase in MSNA occurs in the absence of any detectable decrease in the aortic pressure stimulus to the sinoaortic baroreceptors. (3) In normal subjects, the large increase in MSNA during a high level of LBNP is dependent on a decrease in arterial pressure and can be dissociated from the decrease in central venous pressure. Taken together, these findings suggest that ventricular mechanoreceptors play a small role and sinoaortic baroreceptors a large role in the reflex control of the human skeletal muscle circulation during orthostatic stress.

Role of Ventricular Mechanoreceptor Reflexes

Because of the elevated baseline levels of MSNA in heart transplants, as reported previously, we used four different methods of expressing the LBNP-induced changes in sympathetic activity: bursts per minute, bursts per 100 heart beats, mean burst amplitude, and integrated activity. Regardless of the method used to

FIG 3. Original recordings from a representative normal subject and a representative heart transplant (Tx) recipient showing effects of lower body negative pressure (LBNP) at −40 mm Hg on forearm blood flow and blood pressure. This level of LBNP caused sizeable decreases in forearm blood flow in both subjects, as shown by the decreases in the slopes of the plethysmographic tracings. During LBNP, diastolic blood pressure increased slightly in the normal subject but decreased slightly in the heart Tx recipient.

FIG 4. Patient-specific data showing the increases in forearm vascular resistance during lower body negative pressure at −40 mm Hg plotted as a function of time elapsed between heart transplantation and the experimental study. The solid lines connect data from two patients who were studied at two different points in time. The dashed line indicates the 6-month point. Open squares indicate the patients initially studied 2 to 3 months after transplantation. Filled squares indicate the patients studied more than 6 months after transplantation. The vasoconstrictor responses were much smaller when patients were studied within the first 2 to 3 months after transplantation than when studied more than 6 months after transplantation.
express MSNA, the present data demonstrate that during simulated orthostatic stress the neural stimulus to vasoconstriction in the skeletal muscle bed clearly is not attenuated after heart transplantation. This conclusion is strengthened by the additional finding that the forearm vasoconstrictor responses to LBNP were also well preserved after heart transplantation.

These observations would appear to come into conflict with previous data indicating that the forearm vasoconstrictor and plasma norepinephrine responses to graded LBNP were greatly attenuated after heart transplantation. Although many factors may be involved in causing these conflicting results, one such factor may be the time elapsed between heart transplantation and the experimental study, which was considerably shorter in the two previous studies than in ours (1 to 12 vs 1 to 46 months). By studying patients within the first 2 to 3 months after transplantation, we were able to replicate the previously reported observations, suggesting that attenuated forearm vasoconstriction is not a specific effect of ventricular deafferentation but rather is a nonspecific effect, perhaps related to recent congestive heart failure, major surgery, or some other unidentified factor.

However, our data should not be interpreted as suggesting that ventricular mechanoreceptors play no role in causing reflex sympathetic activation and vasoconstriction in the peripheral circulation during orthostatic stress. Indeed, the observation that MSNA did not increase in our heart transplant recipients when the mild LBNP was repeated while clamping blood pressure experimentally is consistent with the view that ventricular afferents normally do contribute to the rather small increase in MSNA during mild nonhypotensive LBNP. This interpretation is contingent on the assumption that in normal humans mild levels of LBNP do not cause even a subtle reduction in the arterial pressure stimulus to the sinoaortic baroreceptors. We provided further support for this assumption using a high-fidelity Millar catheter placed in the aortic arch.

Whereas mean arterial pressure did not fall significantly during even a high level of LBNP in the two previous studies and in our patients studied within the first 3 months after transplantation, in our remaining heart transplant recipients there was a small but statistically significant exaggeration of the normal decreases in arterial pressure during both simulated and actual orthostatic stress. Although the underlying mechanism is unknown, the possibilities include (1) elimination of the normal baroreflex increase in heart rate due to efferent denervation of the cardiac allograft, (2) an excessive orthostatic fall in cardiac output due to diastolic dysfunction of the cardiac allograft, and (3) impaired reflex vasoconstriction in vascular beds other than in skeletal muscle due to differential effects of ventricular mechanoreceptors on the regulation of sympathetic outflow to various organs. Regardless of the precise explanation, the data demonstrate that the ventricular deafferentation resulting from heart transplantation causes only a minor impairment in the orthostatic regulation of blood pressure and does not cause symptomatic orthostatic hypotension.

Decreases in arterial pressure and corresponding increases in MSNA during orthostatic stress might have been spuriously augmented by subclinical hypovolemia due to lingering effects of recently discontinued diuretic therapy. This possibility is unlikely because (1) venous pooling produced comparable decreases in cardiac dimensions in patients and controls; (2) many of our patients had never received diuretic therapy, and all patients were receiving prednisone and cyclosporine, which have been shown to increase plasma volume and (3) in a subset of patients, acute volume expansion did not attenuate the decreases in arterial pressure or increases in MSNA evoked by LBNP.

Another possible explanation for preserved reflex sympathetic activation in heart transplant recipients might be that the cardiac allografts underwent progressive reinnervation over time, leading to gradual restoration of ventricular baroreflex control of sympathetic activity. That explanation, however, is improbable. In our patients, the magnitude of the sympathetic response to LBNP did not vary as a function of time elapsed after transplantation. Although there is some evidence for partial reinnervation of human cardiac allografts, the reinnervation is not detectable for the first several years after transplantation, is not evident in the majority of patients, and is evident mainly for intrinsic, rather than extrinsic, cardiac nerves. Further evidence for the persistence of ventricular deafferentation after orthotopic heart transplantation is the persistent loss of bradycardia and the forearm vasodilator response to intracoronary ionic contrast material, which is a potent stimulus to ventricular afferents.

During orthotopic cardiac transplantation, ventricular afferents are severed, but afferents arising from the recipient atrial remnant, venoatrial junctions, lungs, and carotid sinus and aortic arch baroreceptors are left intact. In our heart transplant recipients, therefore, unloading of residual cardiopulmonary afferents might have contributed to the observed increases in MSNA during orthostatic stress. Although we cannot completely exclude any role for such afferents in these experiments, removal of the vast majority of cardiopulmonary afferents by combined heart-lung transplantation also apparently fails to impair forearm vasoconstriction during LBNP and fails to impair systemic vasoconstriction during upright tilt.
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A Ventricular Rate (beats/min)

A lnnerVated Atrial Rate (beats/min)

A Mean Burst Amplitude (mm)

A Integrated MSNA (units)

Δ MAP (mm Hg)

Δ MAP (mm Hg)

FIG 5. Graphs of summary data comparing sinoaortic baroreflex-mediated changes in ventricular and atrial heart rates and in muscle sympathetic nerve activity (MSNA), the latter expressed both as mean burst amplitude and as integrated activity, during nitroprusside-induced decreases and phenylephrine-induced increases in mean arterial pressure (MAP) in heart transplant (Tx) recipients vs normal control subjects. Data are mean±SEM for seven control subjects and seven heart Tx recipients. Asterisks indicate significant group differences (*P<.05, heart transplant recipients vs normal subjects) in the slope of the lines relating changes in heart rate or MSNA to changes in arterial pressure. These alterations in arterial pressure had almost no effect on donor ventricular heart rates but caused changes in innervated recipient remnant atrial heart rates that were comparable to the increases in sinus rates in the control subjects. Phenylephrine-induced increases in arterial pressure evoked comparable decreases in MSNA in patients and control subjects. However, nitroprusside-induced decreases in arterial pressure evoked threefold larger increases in mean burst amplitude in Tx recipients vs normal control subjects (P<.05) but comparable increases in integrated MSNA (bursts per minute times mean burst amplitude).

TABLE 5. Responses in Normal Subjects (n=9) to Application of Graded Lower Body Negative Pressure Alone and With Concomitant Infusion of Phenylephrine

<table>
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<tr>
<th></th>
<th>Baseline</th>
<th>-10 mm Hg</th>
<th>-15 mm Hg</th>
<th>-20 mm Hg</th>
<th>-30 mm Hg</th>
<th>-40 mm Hg</th>
<th>-45 mm Hg</th>
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<tr>
<td>Systolic pressure (mm Hg)</td>
<td>129±3</td>
<td>128±3</td>
<td>127±2</td>
<td>126±3</td>
<td>125±3*</td>
<td>122±2*</td>
<td>128±2</td>
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<td>Diastolic pressure (mm Hg)</td>
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<td>62±2</td>
<td>61±2</td>
<td>61±2</td>
<td>59±2</td>
<td>56±2*</td>
<td>62±2</td>
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<td>Mean pressure (mm Hg)</td>
<td>85±2</td>
<td>84±2</td>
<td>84±2</td>
<td>83±2</td>
<td>80±1*</td>
<td>78±1*</td>
<td>83±2</td>
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<tr>
<td>Central venous pressure (mm Hg)</td>
<td>4.5±0.4</td>
<td>2.2±0.3*</td>
<td>0.8±0.6*</td>
<td>-0.8±0.6*</td>
<td>-2.1±0.6*</td>
<td>-3.0±0.5*</td>
<td>-2.3±0.5*</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>69±2</td>
<td>66±2</td>
<td>68±2</td>
<td>71±3</td>
<td>75±3*</td>
<td>80±3*</td>
<td>72±5</td>
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<td>MSNA Burst per minute</td>
<td>25±3</td>
<td>28±3</td>
<td>33±3*</td>
<td>36±2*</td>
<td>43±3*</td>
<td>50±3*</td>
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<td>MSNA Burst per 100 beats</td>
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<td>51±3*</td>
<td>57±4*</td>
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</tr>
<tr>
<td>MSNA Integrated activity (units)</td>
<td>406±71</td>
<td>450±61</td>
<td>583±65*</td>
<td>748±67*</td>
<td>1022±86*</td>
<td>1231±111*</td>
<td>468±83</td>
</tr>
</tbody>
</table>

LBNP, lower body negative pressure; PE, phenylephrine; bpm, beats per minute; MSNA, muscle sympathetic nerve activity. Values are mean±SEM.

*P<.05 vs baseline.
Role of Sinusoidal Baroreflexes

Our data provide multiple lines of evidence that in heart transplant recipients unloading of sinusoidal, rather than cardiopulmonary, baroreceptors is the primary mechanism causing sympathetic activation in skeletal muscle during hypotensive orthostatic stress. During both nitroprusside-induced decreases and phenylephrine-induced increases in blood pressure in the patients, sinusoidal baroreflex control of remnant atrial heart rate was intact, which confirms and extends the conclusion of a previous study.34 Sinusoidal baroreflex control of MSNA also was not attenuated and even appeared to be augmented. The latter finding is consistent with the well-established concept of an inhibitory interaction between cardiopulmonary and sinusoidal baroreflexes such that reduction in cardiopulmonary afferent input augments the gain of the sinusoidal baroreflex.35-39 In addition, the transplant recipients' larger than normal decreases in arterial pressure during orthostatic stress were accompanied by proportionally large increases in innervated atrial remnant heart rates, indicating a large reduction in sinusoidal baroreflex restraint on sinus node function. Finally, the increases in both atrial remnant heart rates and MSNA during mild LBNP were greatly attenuated by concomitant infusion of phenylephrine, a maneuver that offset the narrowing of arterial pulse pressure and thus presumably the unloading of sinusoidal baroreceptors.

On the basis of our findings in heart transplant recipients, we performed additional experiments with phenylephrine to reevaluate the role played by sinusoidal, relative to cardiopulmonary, baroreflexes in the integrated regulation of MSNA during orthostatic stress in humans with intact ventricular afferents. Previously, concomitant carotid baroreceptor stimulation with neck suction was found in normal humans to abolish the increases in heart rate and splanchnic vascular resistance during LBNP at −40 mm Hg but to have no effect on the vasoconstrictor response in the forearm. Thos data were interpreted to suggest that, although sinusoidal baroreflexes normally are engaged during this high level of LBNP, they are much less important than cardiopulmonary baroreflexes in causing vasoconstriction in the peripheral circulation. That interpretation was supported by the finding that during a ramp increase in LBNP the increase in forearm vascular resistance mirrored the decrease in central venous pressure, with most of the reflex response preceding a detectable decrease in arterial pressure.8

In contrast to forearm vascular resistance, MSNA in normal subjects displayed a very different pattern of response to a ramp increase in LBNP, with only a small fraction of the peak reflex response preceding a decrease in arterial pressure and most of the increase in sympathetic activity occurring during the hypotensive levels of LBNP. These findings, which replicate those from several previous microneurographic studies,9,40,41 suggest that the muscle sympathetic nerve response to graded LBNP is not as closely associated with central venous pressure as has been previously assumed.8,18,42

Furthermore, the ability to measure sympathetic activity, not just vascular resistance, permitted the use of a vasoconstrictor drug as an experimental approach to offset simultaneously the unloading of both carotid and aortic baroreceptors during LBNP. This experiment allowed us to dissociate MSNA from central venous

---

**Table 6. Hemodynamic Responses in Central Aorta to Lower Body Negative Pressure**

<table>
<thead>
<tr>
<th></th>
<th>LBNP</th>
<th>Baseline</th>
<th>−5 mm Hg</th>
<th>−10 mm Hg</th>
<th>−15 mm Hg</th>
<th>−20 mm Hg</th>
<th>−40 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td></td>
<td>125±3</td>
<td>125±3</td>
<td>124±2</td>
<td>123±2</td>
<td>120±3*</td>
<td>115±2*</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td></td>
<td>86±5</td>
<td>86±5</td>
<td>86±4</td>
<td>85±5</td>
<td>86±5</td>
<td>85±4</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td></td>
<td>99±4</td>
<td>99±4</td>
<td>99±3</td>
<td>98±3</td>
<td>97±4</td>
<td>95±3*</td>
</tr>
<tr>
<td>dP/dt max (mm Hg/sec)</td>
<td></td>
<td>676±74</td>
<td>677±74</td>
<td>665±80</td>
<td>653±80</td>
<td>627±80*</td>
<td>582±80*</td>
</tr>
</tbody>
</table>

LBNP, lower body negative pressure. Values are mean±SEM for eight experiments performed in four normal subjects.

*P<.05 vs baseline.
pressure during LBNP and demonstrate a strong association with arterial pressure. The results suggest that a large portion of the reflex increase in MSNA during hypotensive LBNP can be explained by unloading of sinoaortic baroreceptors, with the residual phenylephrine-resistant increase in MSNA being consistent with a smaller role for cardiopulmonary afferents. In light of recent evidence that aortic baroreceptors may be more important than carotid baroreceptors in the reflex regulation of MSNA during pharmacological decreases in arterial pressure in normal humans, our present microneurographic data indicate that the earlier plethysmographic studies, which were limited methodologically to study the influence of only the carotid baroreceptors, underestimated the overall contribution of the sinoaortic (i.e., carotid plus aortic) baroreflex to the integrated control of muscle sympathetic vasoconstrictor drive during hypotensive orthostatic stress.

In our experiments, the phenylephrine dose was carefully titrated to restore arterial pressure to the baseline value but not to exceed it. However, two other aspects of this experimental design could overestimate the importance of the sinoaortic baroreflex and underestimate the importance of the cardiopulmonary mechanoreflexes. First, in anesthetized rabbits, prolonged intravenous infusion of phenylephrine produces a central sensitization of the sinoaortic baroreflex, causing sympathetic activity to remain suppressed for several minutes after resolution of the phenylephrine-induced increase in blood pressure. Similarly, in conscious humans, muscle sympathetic activity, but not heart rate, can remain decreased for several minutes after resolution of a phenylephrine-induced increase in blood pressure. However, we found no evidence for such sensitization of the sinoaortic baroreflex in our experiments involving either heart transplant recipients or normal control subjects. When we continued the LBNP after discontinuing the phenylephrine infusion, blood pressure, heart rate, and MSNA all returned promptly to the preinfusion values with no lag in the recovery of the MSNA response.

Second, because sinoaortic and cardiopulmonary baroreflexes interact, it is not surprising that the gain of the sinoaortic baroreflex was greater than normal in heart transplant recipients with ventricular deafferentation. In normal humans, this interaction should cause the gain of the cardiopulmonary mechanoreflexes to be greater when sinoaortic and cardiopulmonary baroreceptors are unloaded simultaneously, as occurs normally during orthostatic stress, than when the normal unloading of sinoaortic baroreceptors is prevented with phenylephrine. This effect of phenylephrine should be emphasized in light of experimental evidence for hysteresis in sinoaortic baroreflex, such that at a given level of arterial pressure the baroreceptors are stimulated to a greater degree when pressure is raised, as with phenylephrine, than when it is lowered.

Because of these considerations, the present data do not permit a precise quantitation of the relative contributions of sinoaortic baroreflexes and ventricular mechanoreflexes in this setting. Nevertheless, the data strongly suggest that sinoaortic baroreflexes are much more important and ventricular baroreflexes are much less important than previously thought in causing reflex sympathetic activation and vasoconstriction in the human skeletal muscle circulation during orthostatic stress.

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References


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T N Jacobsen, B J Morgan, U Scherrer, S F Vissing, R A Lange, N Johnson, W S Ring, P S Rahko, P Hanson and R G Victor

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