Hypovolemia and neurovascular control during orthostatic stress

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Kimmerly, Derek S., and J. Kevin Shoemaker. Hypovolemia and neurovascular control during orthostatic stress. Am J Physiol Heart Circ Physiol 282: H645–H655, 2002. First published September 27, 2001; 10.1152/ajpheart.00535.2001.—Humans exposed to real or simulated microgravity experience decrements in blood pressure regulation during orthostatic stress that may be related to autonomic dysregulation and/or hypovolemia. We examined the hypothesis that hypovolemia, without the deconditioning effects of bed rest or spaceflight, would augment the sympathoneural and vasomotor response to graded orthostatic stress. Radial artery blood pressure (tonometry), stroke volume (SV), brachial blood flow (Doppler ultrasound), heart rate (electrocardiogram), peroneal muscle sympathetic nerve activity (MSNA; microneurography), and estimated central venous pressure (CVP) were recorded during five levels (–5, –10, –15, –20 and –40 mmHg) of randomly assigned lower body negative pressure (LBNP) (n = 8). Forearm (FVR) and total peripheral vascular resistance (TPR) were calculated. The test was repeated under randomly assigned placebo (normovolemia) or diuretic (spironolactone: 100 mg/day, 3 days) (hypovolemia) conditions. The diuretic produced an ~16% reduction in plasma volume. Compared with normovolemia, SV and cardiac output were reduced by ~12% and ~10% at baseline and during LBNP after the diuretic. During hypovolemia, there was an upward shift in the ΔMSNA/ΔCVP, ΔFVR/ΔCVP, and ΔTPR/ΔCVP relationships during 0 to –20 mmHg LBNP. In contrast to normovolemia, blood pressure increased at –40 mmHg LBNP during hypovolemia due to larger gains in the ΔMSNA/ΔCVP and ΔTPR/ΔCVP relationships. It was concluded that acute hypovolemia augmented the neurovascular component of blood pressure control during moderate orthostasis, effectively compensating for decrements in SV and cardiac output.

baroreflex; muscle sympathetic nerve activity; Doppler ultrasound; lower body negative pressure; vascular resistance; spironolactone

EXPOSURE TO REAL OR SIMULATED MICROGRAVITY leads to cardiovascular deconditioning with the associated reductions in blood pressure regulation during orthostatic stress. The effect of this deconditioning on baroreflex neurovascular control in humans is not known, and the pathophysiology of postflight difficulties in blood pressure control remains a focus of debate. To maintain adequate blood pressure and cerebral perfusion during orthostatic stress, reflex adjustments occur to increase heart rate (HR) and peripheral vasoconstriction to compensate for a decreased venous return and stroke volume (SV). Primary contributors to the diminished ability to maintain blood pressure in many individuals after spaceflight or bed rest are believed to include reductions in plasma volume (PV) (3, 7, 10, 11), diminished baroreflex control of HR (10, 21), and/or vascular resistance (36, 48). From these findings, two separate hypotheses have been proposed to explain difficulties in postural blood pressure control after spaceflight or bed rest.

The first hypothesis recognizes the positive correlation between the duration of microgravity exposure and the degree of PV reduction reaching 12–15% or 350–500 ml (3). Hypovolemia leads to larger decreases in both venous return and SV during an orthostatic stress, subsequently compromising blood pressure control (25, 40). Evidence challenging this hypothesis comes from studies that have used countermeasures such as isotonic fluid loading (7), lower body negative pressure (LBNP) protocols (3), and/or intense bouts of endurance exercise (12) to restore PV to preflight levels before reentry without decreasing the incidence of postflight orthostatic intolerance (6, 25).

The second hypothesis states that difficulties in blood pressure control subsequent to cardiovascular deconditioning are related to inadequate increases in autonomic nervous system control of peripheral vascular resistance in response to decreases in cardiac filling pressure (3, 5). Butler et al. (8) observed that 4 h of head-down tilt bed rest produced an increase in the incidence of orthostatic intolerance without concurrent reductions in PV, suggesting that factors unrelated to circulating blood volume were major contributors to the decreased orthostatic blood pressure regulation. Recently, Buckey et al. (6) reported that a major distinction between returning astronauts who could complete a 10 min stand test versus those who could not was an ability to augment the increase in total peripheral resistance (TPR) upon standing despite similar reductions in PV and SV in all individuals. Evidence of smaller increases in sympathetic nerve activity in

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those individuals who became presyncopal during head-up tilt after 14 days of bed rest (47) support the findings of Buckey et al. (6). In contrast, some studies have argued against autonomic adjustments after microgravity-induced cardiovascular deconditioning. On the basis of measures of TPR and blood pressure, Baisch et al. (2) concluded that the cardiovascular deficits during LBNP after spaceflight durations of 8–20 days were the consequence of a fluid deficit rather than to changes in autonomic function. In addition, sympathetic responses to mild (39) and severe (31) orthostatic stress were not altered after 18 and 120 days of head-down bed rest, respectively.

However, it is possible that the normal sympathetic adjustments observed in these latter studies (31, 39) were a compensatory response to concurrent reductions in PV. This possibility is supported by evidence that a normal post bed rest sympathetic response was associated with apparently normal orthostatic tolerance (31, 47). In addition, acute diuretic-induced hypovolemia has been shown to augment blood pressure responses to the Valsalva maneuver (21, 32) and forearm vasomotor responses to reductions in central venous pressure (CVP) (14, 32, 51) Thus we hypothesized that moderate hypovolemia (i.e., the range observed with bed rest or spaceflight), in the absence of cardiovascular deconditioning stimuli, provides a protective mechanism that increases integrated (i.e., cardiopulmonary and arterial) baroreflex-mediated vasomotor control during postural stress, effectively compensating for reductions in SV. Thus the purpose of the present study was to determine whether acute hypovolemia alone enhances the neurovascular response to simulated orthostatic stress. To this end, muscle sympathetic nerve activity (MSNA) was measured during graded LBNP with and without diuretic-induced PV reductions. Measures of estimated CVP, forearm blood flow (FBF), and cardiac output were obtained to examine the effects of hypovolemia on systemic and peripheral vascular responses.

METHODS

Participants. Eight healthy normotensive males volunteered for the present study. Each participant provided signed consent to the experimental procedures, which were approved by the University of Western Ontario Review Board for Health Science Research Involving Human Subjects.

The participants were 23 ± 2 (mean ± SD) yr of age, with average heights and weights of 178 ± 6 cm and 85 ± 6 kg, respectively. All participants refrained from nicotine and caffeinated and alcoholic beverages for a minimum of 24 h before testing. They were instructed to maintain their normal food and fluid intake between all testing sessions. All participants arrived to the laboratory after a 12-h fast. A standardized carbohydrate snack and beverage was then given before each experiment in an attempt to normalize hydration status. All participants were asked to void their bladder before instrumentation.

Experimental design. Each participant reported to the laboratory on three separate occasions. During the first familiarization visit, each participant performed a portion of the protocol including the noninvasive components of data acquisition. The two subsequent testing sessions were separated by a minimum of 1 wk and occurred at the same time of day. One session occurred after the oral administration of the diuretic and the other after a placebo was taken. The diuretic used in the present study was the aldosterone receptor antagonist spironolactone (Aldactone). It was given in capsule form at a dose of 100 mg/day for 3 days. The order of diuretic versus placebo testing was randomly assigned to each participant.

Lower body negative pressure protocol. The participants began by lying supine on the laboratory bed with their legs and hips sealed in a LBNP chamber. The LBNP protocol included an initial 10-min period of baseline rest followed by 5-min applications of negative pressure at −5, −10, −15, and −20 mmHg. These low levels of LBNP have traditionally been used to optimize examination of cardiopulmonary baroreceptor activation (14, 40, 51). A 5-min rest period was positioned between each of these levels of LBNP. The order of LBNP was randomized between participants, but the same order of LBNP was used for both experiments for a given subject. After the final LBNP level, chamber pressure was immediately reduced to −40 mmHg for an additional 5 min. This stimulus was used to examine the effect of hypovolemia on the integrated cardiopulmonary and arterial baroreflex response to moderate cardiovascular stress.

Data collection. HR was determined by standard three-lead electrocardiogram techniques. The electrocardiogram tracing was continuously monitored for abnormal heart patterns. No arrhythmias were observed in any participant either at rest or during LBNP. Continuous arterial blood pressure was monitored over the radial artery using a tonometric sensor (Pilot, Colin Medical Instruments; San Antonio, TX) (55). The tonometric sensor contains piezoresistive pressure transducers that are held against the skin and tissues above the artery. With the artery partially flattened, the tonometric sensor detects continuous pressure changes from the wall of the artery. The tonometric system was periodically calibrated during each baseline period using pressures derived from a self-inflating upper arm cuff.

An estimate of CVP was determined from the antecubital venous pressure in the dependent arm using a disposable transducer (model PX272, Baxter) connected and referenced to the tip of a 20-gauge catheter, which was inserted in an anterograde fashion into the vein after the arm was lowered below the heart. The vertical height from the transducer to the right atrium was measured to assess the hydrostatic column and thereby determine an estimate of CVP.

At the start of each experiment and after ~15-min accommodation to the supine position, a 1-ml blood sample was obtained from the antecubital vein for hematocrit determination. In one subject, the hematocrit was determined from samples obtained in the seated position. The PV change was calculated using van Beaumont’s equation (52).

Both aortic root (2.5-MHz transducer, parasternal long-axis view) and brachial artery (7.5-MHz transducer) diameter measurements were made before the start of each experimental session from a frozen two-dimensional B-mode image (GEVingmed System Five) at end diastole. Images of the inferior vena cava (IVC) (2.5 MHz) were obtained during expiration during all steady-state periods of baseline and LBNP, with diameter measures made where this vessel intersects with the hepatic vein.

Measurement of brachial artery mean blood velocity was obtained using a flat 4.0-MHz pulsed Doppler probe (model 500 M, Multigon; Yonkers, NY) with an insonation angle of 45°. SV velocity in the ascending aorta was obtained from the suprasternal notch using a hand-held 2.0-MHz pulsed
wave probe (model CFM750, Vingmed). An insonation angle of 20° was assumed for the ascending aortic velocity calculations.

Multiunit recordings of postganglionic sympathetic nerve activity were obtained from the common peroneal nerve (16, 17) with 35-mm-long tungsten microelectrodes with a shaft diameter of 200 μm that tapered to an uninsulated tip of 1–5 μm. A reference electrode was inserted subcutaneously 1–3 cm from the recording electrode. Neuronal activity was amplified 1,000 times by a preamplifier and 50–100 times by a variable-gain isolated amplifier. The signal was band-pass filtered with a bandwidth of 700–2,000 Hz and was then rectified and integrated to obtain a mean voltage neurogram with a time constant of 0.1 s. A MSNA site was confirmed by manipulating the microelectrode until the characteristic pulse-synchronous burst pattern was observed that increased in frequency during a voluntary apnea but did not change in response to arousal or produce skin paresthesias (17).

**Data analysis.** Analog signals for blood pressure, CVP, MSNA, mean blood velocity, SV velocity (sampled at 200 Hz), and electrocardiograms (sampled at 400 Hz) were collected with an on-line data acquisition and analysis system (PowerLab, ADInstruments; Castle Hill, New South Wales, Australia). Approximately 60 s of continuous data were obtained from minutes 3 to 5 of each level of LBNP and the intervening baseline periods and averaged for analysis.

FBF and cardiac output were calculated as the product of vessel cross-sectional area and the corresponding blood velocity values, accounting for changes in cardiac period. The mean systolic and diastolic blood pressure values over the final 2 min of each baseline and LBNP period were used to calculate mean arterial pressure (MAP; equal to diastolic pressure plus one-third of pulse pressure). TPR and forearm vascular resistance (FVR) were calculated as (MAP – CVP)/FBF and (MAP – CVP)/cardiac output, respectively.

Only bursts of MSNA activity with a 2:1 or greater signal-to-noise ratio were considered for analysis. MSNA activity was measured for amplitude per burst and frequency per minute during each period of baseline and LBNP level. Total MSNA activity was calculated as the sum of analog burst amplitudes per minute. The sympathetic response was determined by relating the increase in total MSNA during a given level of LBNP to its respective baseline period.

**Statistical analysis.** The effects of LBNP and PV on hemodynamic and MSNA variables were analyzed using a repeated-measures two-way ANOVA. When significant main effects were observed, Tukey’s post hoc analysis was performed to estimate differences among means. Probability levels during multiple-point-wise comparisons were corrected using Bonferroni’s approach.

Cardiopulmonary baroreflex control was estimated from stimulus-response curves between changes in CVP versus TPR, FVR, and %MSNA using data from the −5 to −20 mmHg of LBNP periods. Individual slope and y-intercepts were determined for each subject for all relationships. Mean curves were then generated from the individual slope and y-intercept values. The integrated baroreflex response was compared using the same relationships but from data obtained only at −40 mmHg LBNP. Changes in the slopes and y-intercepts between hypovolemia and normovolemia for all CVP relationships were determined using two-tailed paired t-tests. Statistical significance in all comparisons was set at P < 0.05. Values are presented as means ± SE.

**RESULTS**

Technical limitations prevented collection of complete data from all study segments in all subjects. Specifically, during hypovolemia, complete CVP data were collected from six subjects at −5, −10, and −20 mmHg LBNP and from four subjects only at −15 mmHg. Otherwise, complete hemodynamic and MSNA data were obtained from seven subjects at −10 and −20 mmHg LBNP during hypovolemia and from six subjects at −15 mmHg LBNP (normovolemia) and −40 mmHg LBNP (hypovolemia).

**Baseline and LBNP responses.** There was no effect of time or repeated LBNP periods on cardiovascular hemodynamic or sympathetic variables measured in the intervening rest segments (Table 1). Moreover, the average of the intervening rest periods was not different than the pre-LBNP baseline data for all variables (Table 1). Spironolactone administration increased hematocrit from 43.6 ± 0.6% to 47.8 ± 0.5% (P < 0.05), resulting in a 15.5 ± 1.7% (P < 0.05) reduction in resting PV (range = 8–20% reduction). This hypovolemia was associated with a 1.4-mmHg reduction in baseline CVP (P < 0.05), an 11.7% reduction in SV (P < 0.05), and corresponding reductions in cardiac output and FBF (P < 0.05) (Table 1). Baseline MAP, HR, and IVC diameter were not altered by spironolactone administration. Subsequently, baseline FVR and TPR were augmented in association with a ~23% increase in MSNA (all P < 0.05) (Table 1).

**Effects of LBNP.** Compared with baseline, CVP was reduced during LBNP, becoming statistically significant at −40 mmHg LBNP (Fig. 1). The diuretic-induced reductions in CVP observed at rest were sustained during LBNP (main effect of spironolactone, P < 0.05) (Figs. 1 and 2). There was no difference in the IV response to LBNP between conditions (Fig. 1). During normovolemia, HR increased by 5 and 13 beats/min at −20 and −40 mmHg, respectively (P < 0.05; Fig. 3). Hypovolemia did not change the HR response, with increases of 9 ± 3 and 16 ± 5 beats/min at −20 and −40 mmHg LBNP, respectively (Fig. 3).

SV decreased, with LBNP becoming statistically significant by −20 mmHg LBNP (P < 0.05; Fig. 3). The reduction in baseline SV in hypovolemia was sustained throughout LBNP (main effect of condition, P < 0.05; Fig. 3). Specifically, during normovolemia, SV was reduced from a baseline value of 103 ± 11 to 80 ± 11 and 69 ± 9 ml/beat at −20 and −40 mmHg LBNP, respectively. Hypovolemic levels decreased from 91 ± 11 to 67 ± 7 and 54 ± 5 ml/beat at −20 and −40 mmHg, respectively.

Cardiac output was reduced with −40 mmHg of LBNP (P < 0.05; Fig. 3), decreasing from 6,550 ± 755 to 5,208 ± 643 ml/min during normovolemia and from 5,905 ± 707 to 4,416 ± 449 ml/min during hypovolemia (main effect of spironolactone, P < 0.05).

Original tracings of arterial blood pressure, CVP, and MSNA recordings at baseline and −40 mmHg LBNP for a single individual are shown in Fig. 2. These data highlight the reduction in CVP and increase in
Table 1. Baseline (0 mmHg LBNP) cardiovascular and sympathetic variables before (normovolemia) and after (hypovolemia) diuretic administration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (0 mmHg LBNP)</th>
<th>Before −5 mmHg LBNP</th>
<th>Before −10 mmHg LBNP</th>
<th>Before −15 mmHg LBNP</th>
<th>Before −20 mmHg LBNP</th>
<th>Mean</th>
<th>Pre-LBNP</th>
</tr>
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<tr>
<td>HR, beats/min</td>
<td>Normovolemia</td>
<td>64 ± 2</td>
<td>64 ± 2</td>
<td>64 ± 2</td>
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<td>64 ± 0.3</td>
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<td></td>
<td>Hypovolemia</td>
<td>64 ± 2</td>
<td>65 ± 2</td>
<td>66 ± 2</td>
<td>65 ± 0.3</td>
<td>65 ± 2</td>
<td></td>
</tr>
<tr>
<td>SV, ml/beat</td>
<td>Normovolemia</td>
<td>104 ± 11</td>
<td>101 ± 10</td>
<td>103 ± 13</td>
<td>104 ± 10</td>
<td>103 ± 1</td>
<td>103 ± 1</td>
</tr>
<tr>
<td></td>
<td>Hypovolemia</td>
<td>90 ± 12</td>
<td>91 ± 11</td>
<td>92 ± 11</td>
<td>89 ± 10</td>
<td>91 ± 1</td>
<td>89 ± 12</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>Normovolemia</td>
<td>89 ± 4</td>
<td>87 ± 6</td>
<td>88 ± 4</td>
<td>87 ± 4</td>
<td>88 ± 1</td>
<td>88 ± 2</td>
</tr>
<tr>
<td></td>
<td>Hypovolemia</td>
<td>85 ± 3</td>
<td>86 ± 4</td>
<td>87 ± 4</td>
<td>85 ± 4</td>
<td>86 ± 1</td>
<td>86 ± 2</td>
</tr>
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<td>TPR, a.u.</td>
<td>Normovolemia</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>13 ± 3</td>
</tr>
<tr>
<td></td>
<td>Hypovolemia</td>
<td>16 ± 2</td>
<td>16 ± 2</td>
<td>17 ± 2</td>
<td>16 ± 2</td>
<td>16 ± 2*</td>
<td>15 ± 2*</td>
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<tr>
<td>FBF, ml/beat</td>
<td>Normovolemia</td>
<td>37 ± 6</td>
<td>39 ± 6</td>
<td>39 ± 6</td>
<td>37 ± 6</td>
<td>38 ± 0.5</td>
<td>39 ± 3</td>
</tr>
<tr>
<td></td>
<td>Hypovolemia</td>
<td>29 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 2*</td>
<td>27 ± 3*</td>
<td>27 ± 3*</td>
</tr>
<tr>
<td>FVR, a.u.</td>
<td>Normovolemia</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>2.6 ± 0.1</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
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<td>Hypovolemia</td>
<td>3.1 ± 0.3</td>
<td>3.3 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>3.3 ± 0.5</td>
<td>3.3 ± 0.1</td>
<td>3.2 ± 0.2*</td>
</tr>
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<td>CVP, mmHg</td>
<td>Normovolemia</td>
<td>5.5 ± 0.7</td>
<td>5.5 ± 0.8</td>
<td>5.5 ± 0.7</td>
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<td>5.6 ± 1</td>
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<tr>
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<td>3.9 ± 0.4</td>
<td>4.0 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>4.1 ± 0.4</td>
<td>4.0 ± 0.1</td>
<td>3.8 ± 1*</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>Normovolemia</td>
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<td>21 ± 2</td>
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<td>23 ± 2</td>
<td>22 ± 1</td>
<td>22 ± 2</td>
</tr>
<tr>
<td></td>
<td>Hypovolemia</td>
<td>29 ± 3</td>
<td>27 ± 3</td>
<td>27 ± 3</td>
<td>26 ± 3</td>
<td>27 ± 1*</td>
<td>28 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pre-LBNP, baseline period before first administered level of lower body negative pressure; HR, heart rate; SV, stroke volume; MAP, mean arterial pressure; TPR, total peripheral resistance; FBF, forearm blood flow; FVR, forearm vascular resistance; IVC, inferior vena cava diameter; CVP, central venous pressure; a.u., arbitrary units. *P < 0.05, hypovolemia vs. normovolemia.

arterial blood pressure with hypovolemia in association with augmented MSNA, particularly at −40 mmHg LBNP. Compared with baseline values, MAP did not change in either condition on going from −5 to −20 mmHg of LBNP (Fig. 4). During normovolemia, MAP decreased by 5 ± 2 mmHg from baseline to −40 mmHg (P < 0.05) (Figs. 2 and 4). In contrast, MAP increased by 6 ± 3 mmHg from baseline to −40 mmHg LBNP during hypovolemia (P < 0.05) (Figs. 2 and 4).

Graded levels of LBNP produced consistent and progressive increases in the %MSNA (Fig. 4). Compared with normovolemia, the %MSNA was augmented in hypovolemia (main effect of spironolactone, P < 0.05). Specifically, the %MSNA at −5 to −40 mmHg increased by 127 ± 12% during normovolemia and by 137 ± 15% in hypovolemia.

The diuretic-induced augmentation of TPR was also maintained throughout LBNP (main effect of spironolactone, P < 0.05). Compared with rest (0 mmHg), TPR was elevated at −20 and −40 mmHg in both trials (Fig. 4) (P < 0.05). TPR at −40 mmHg during hypovolemia (23 ± 3 arbitrary units (a.u.)) was greater than that during normovolemia (18 ± 3 a.u.) (P < 0.05).

The spironolactone-induced increase in baseline FVR and reduction in FBF were both maintained during all levels of LBNP (main effect of spironolactone, P < 0.05; Fig. 5). FBF was reduced from baseline by −5 ± 3 ml/min (P < 0.05) at −40 mmHg LBNP during normovolemia and by −11 ± 5 ml/min (P < 0.05) during hypovolemia (Fig. 5). The reduction in FBF was due to increases in (P < 0.05) FVR at −40 mmHg during both normovolemia (19 ± 6%, P < 0.05) and hypovolemia (29 ± 10%, P < 0.05) (Fig. 5).

Cardiopulmonary baroreflex response. The absence of CVP data at −15 mmHg did not affect determination of the reflex response. When the slope of the regression lines between −5 and −10 mmHg and −15 and −20 mmHg were compared using the mean data points, no differences were observed (Table 2), thus indicating that the baroreflex response to low levels of LBNP was linear across the range of data.

Average stimulus-response characteristics of the cardiopulmonary baroreflex control of TPR, FVR, and MSNA in normovolemia and hypovolemia are shown in Fig. 6. Values between −5 and −20 mmHg were used to optimize analysis of the cardiopulmonary baroreflex and to facilitate direct comparisons with earlier reports (16, 36). Differences in the slope (ΔTPR/ΔCVP, ΔFVR/ΔCVP, and ΔMSNA/ΔCVP) between normovolemia and hypovolemia were compared after determining the mean slopes generated by all subjects. There were no significant differences found between the mean slopes of the two conditions for any relationship. Mean slopes for normovolemia and hypovolemia, respectively, were as follows: ΔTPR/ΔCVP, −1.72 ± 0.12 vs. −1.54 ± 0.08 a.u.; ΔFVR/ΔCVP, −0.17 ± 0.004 vs. −0.12 ± 0.001 a.u.; and ΔMSNA/ΔCVP, −36 ± 12 vs. −39 ± 16 a.u. It is noteworthy that this approach to developing the mean regression did not affect the outcome. Specifically, there was no difference in the slope of the
relationships when the data were plotted as a regression line through the mean data points. Slope values using this latter method for normovolemia and hypovolemia were as follows: $\Delta\text{TPR}/\Delta\text{CVP}, -1.75 \pm 0.13$ vs. $-1.55 \pm 0.07$ a.u.; $\Delta\text{FVR}/\Delta\text{CVP}, -0.19 \pm 0.005$ vs. $-0.14 \pm 0.002$ a.u.; and $\Delta\%\text{MSNA}/\Delta\text{CVP}, -40 \pm 13$ vs. $-44 \pm 16$ a.u., respectively.

The $y$-intercept for the $\%\text{MSNA}/\Delta\text{CVP}$ increased from $-10.2 \pm 2.2$ a.u. in normovolemia to $34.3 \pm 11.3$ a.u. after diuretic administration ($P < 0.05$). Compared with normovolemia ($-1.0 \pm 0.4$ a.u.), the $y$-intercept for the $\Delta\text{TPR}/\Delta\text{CVP}$ relationship during hypovolemia was $4.0 \pm 0.6$ a.u. ($P < 0.05$). For the $\Delta\text{FVR}/\Delta\text{CVP}$ relationship, the $y$-intercept increased from $0.24 \pm 0.07$ a.u. during normovolemia to $0.37 \pm 0.08$ a.u. during hypovolemia ($P < 0.05$).

**Integrated baroreflex response.** The changes in TPR, FVR, and MSNA at the level of $-40$ mmHg LBNP relative to changes in CVP were examined from five individuals to assess average stimulus-response characteristics of integrated baroreflex cardiovascular control (Fig. 7). Compared with normovolemia ($1.2 \pm 0.2$ a.u.), the $\Delta\text{TPR}/\Delta\text{CVP}$ relationship increased during hypovolemia ($2.6 \pm 0.6$ a.u.) ($P < 0.05$). Similar increases were observed for $\Delta\text{FVR}/\Delta\text{CVP}$ (from $0.11 \pm 0.07$ to $0.63 \pm 0.17$ a.u.) ($P < 0.05$) and $\%\text{MSNA}/\Delta\text{CVP}$ (from $49 \pm 12$ to $96 \pm 25$ a.u.) during normovolemia and hypovolemia, respectively ($P < 0.05$). Thus, for a given decrease in CVP, there were greater increases in TPR, FVR, and MSNA during hypovolemia at $-40$ mmHg LBNP.

**DISCUSSION**

The primary new finding from the current investigation was that acute hypovolemia augmented baseline MSNA, and this effect was sustained during $-5$ to $-40$
The augmented MAP with hypovolemia at −40 mmHg LBNP was related to an upward shift in the %MSNA/ΔCVP response (Fig. 7). This shifted sympathetic response was associated with increases in both peripheral and systemic vascular resistance, which were characterized by corresponding upward shifts in the ΔFVR/ΔCVP and ΔTPR/ΔCVP relationships (Fig. 7). Importantly, these enhanced vasomotor reactions during hypovolemia appeared to be instrumental in reversing the hypotension observed at −40 mmHg during normovolemia and, in fact, augmenting MAP at this level of orthostatic stress (Fig. 4). Therefore, these data support the hypothesis that hypovolemia augments neurovascular control during orthostatic stress. Moreover, it appears that in the absence of direct cardiovascular deconditioning stimuli, such as prolonged bed rest or spaceflight, the hypovolemic adaptation is beneficial for blood pressure control at these levels of orthostatic stress.

**Plasma volume reduction.** The hypovolemia that occurs during microgravity exposure is well documented, with a range of 6–16% (3, 10, 12, 13, 25). Similar levels of hypovolemia have also been reported after head-down tilt bed rest (13, 14, 23, 29) and acute diuretic administration of furosemide (21, 29, 32, 51). PV changes in the current study were calculated based on changes in hematocrit measurements. The 4.2 ± 0.2% increase in hematocrit observed in this study is comparable to a 3.7% change observed after 6 days of head-down tilt bed rest (35) but greater than that observed after 16 days of head-down tilt bed rest (~1%) (4) or 9 days of spaceflight (0.2%) (1). However, despite smaller hematocrit changes in these latter studies, PV reductions measured by direct dilution methods (15% and 17% respectively) were similar compared with the 15.5% reduction in PV induced in the current investigation. Moreover, the reduction in resting supine CVP (1.4 ± 0.2 mmHg) in the present study was within the range reported after head-down tilt bed rest (0.8–2.1 mmHg) (11, 23) and during acute hypovolemia (29, 51).
The IVC diameters (Fig. 1) were unchanged in hypovolemia, suggesting that stress-relaxation of the great thoracic veins did not contribute to the observed reduction in CVP.

Intravenous administration of diuretics such as furosemide (21, 32, 51) have been used previously to examine acute hypovolemic effects on cardiovascular control with the advantage that the reduction in PV occurs over 2–4 h. However, bladder distention and the need for micturition interfere with sympathetic discharge (19) and the comfort of the subject during prolonged protocols. Because patient comfort and stability are paramount for microneurographic recordings, we chose to use the oral diuretic spironolactone, causing a slower rate of hypovolemia. One concern with spironolactone is that this drug has direct vasodilatory actions (46) that may have competed with the constrictor responses. However, it is unlikely that this aspect of spironolactone interfered with the current results because...
cause the expected upward shift in the ΔFVR/ΔCVP relationship (14) was observed (Fig. 6). A second concern with the current approach is that 3 days of diuretic administration could have resulted in as many as 3 days of hypovolemia, leading to altered red blood cell mass and hematocrit values. However, this effect is unlikely because hemoconcentration results in reduced red blood cell production (1).

**Cardiopulmonary baroreflex.** Interpreting the effects of acute hypovolemia on the cardiopulmonary stimulus-response relationship is dependent on the assumption that low levels of LBNP selectively unload cardiopulmonary baroreceptor control of sympathetic outflow and vascular resistance. The low levels of LBNP (0 to −20 mmHg) used in this study have traditionally been used to isolate these low-pressure baroreceptors (22, 33, 54). However, the increased HR response during −20 mmHg observed in the current and other (14, 51) studies suggest that arterial baroreceptor unloading also occurred at this level of orthostatic stress (Fig. 3). Nonetheless, several lines of evidence argue that potential arterial baroreflex activation at −20 mmHg does not interfere with interpretations of cardiopulmonary reflex responses. For example, Thompson et al. (51) administered a 10-mmHg hypotensive stimulus to carotid baroreceptors and observed no additional influence on the FVR response to −15 and −20 mmHg LBNP. Moreover, if arterial baroreceptors were influencing the TPR response at −20 mmHg LBNP through, for example, splanchnic vasoconstriction (30), an augmented TPR response would be expected between the levels of −15 and −20 mmHg compared with the corresponding increases between the lower LBNP levels. This effect was not observed (Table 2), suggesting minimal influence of arterial baroreceptor-mediated vasoconstriction up to −20 mmHg LBNP (40, 41).

In previous studies (14, 51) and in the current study, cardiopulmonary baroreflex sensitivity has been examined using the ΔFVR/ΔCVP relationship (Fig. 5). The upward shift in this relationship after hypovolemia in the present study supports earlier findings (51). However, the nature of the hypovolemic effect in these earlier reports is unclear, with contrasting conclusions regarding whether or not the reflex slope was altered (11, 51). The new information in the current study is the provision of knowledge on the efferent neural component of the reflex together with the subsequent vasomotor responses. The data clarify that hypovolemia per se produces an upward shift in both the efferent neural and vascular components of the cardiopulmonary baroreflex with little change in the operational range of CVP. In contrast, prolonged bed rest, which included reductions in blood volume and baroreflex control, was characterized by a leftward shift in the FVR/CVP relationship (51). On the basis of differential effects of bed rest versus hypovolemia on the FVR/CVP response, Convertino (11) proposed that a downward resetting of the cardiopulmonary baroreflex operating point occurred during bed rest that was unrelated to the hypovolemia, such that the normal response for peripheral vasoconstriction occurred at a lower range of CVP. The current data advance the effect of acute hypovolemia by indicating that the operating point for cardiopulmonary sympathetic vascular control is shifted upward on the same stimulus response curve. That is, the MSNA response for a given change in CVP was as expected. The repeatability of MSNA burst frequency on different days (9, 47, 50) provides confidence in this conclusion.

**Integrated baroreflex response.** An important component of the current study was examination of the integrated cardiovascular reflex response to moderate or-

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**Fig. 7. Integrated baroreflex stimulus-response relationships at −40 mmHg between ΔMSNA, ΔTPR, and ΔFVR and estimated ΔCVP before (normovolemia) and after (hypovolemia) diuretic administration. Values are mean ± SE. *Significant difference (P < 0.05) between conditions.**
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orthostatic stress after acute plasma reductions. The larger increases in FVR and TPR in hypovolemia during −40 mmHg LBNP are consistent with the smaller CVP and larger MSNA levels (Fig. 6). Importantly, the sympathetic and vasoconstrictor response for a given drop in CVP during −40 mmHg LBNP was augmented during hypovolemia. Therefore, hypovolemia produced an increased gain in the systemic vasoconstrictor response to moderate orthostatic stress that counterbalanced the progressive decreases in cardiac output. This is different from the reflex responses observed at lower levels of LBNP, where the slope or gain of the TPR, FVR, and MSNA responses to LBNP was the same in the two trials. Therefore, hypovolemia appears to exert differential effects on the low- versus high-pressure baroreflex responses.

A normal rise in MSNA despite a greater increase in MAP at −40 mmHg LBNP during hypovolemia versus normovolemia (Fig. 4) suggests that arterial baroreflex responses to orthostasis may have been enhanced in this volume-depleted state. Aortic baroreceptors are important in the regulation of muscle sympathetic outflow (44, 45). Therefore, the current data provide a possible mechanism for the increased aortic baroreflex responsiveness observed by Crandall et al. (15) after 15 days of bed rest that also included a 15% reduction in PV. If so, then the increased integrated baroreflex response in the current study may have been influenced by aortic baroreflex resetting.

Because the cardiovascular responses during −40 mmHg LBNP are more closely related to the upright posture than −5 to −20 mmHg LBNP, these data raise the issue that acute hypovolemia may lead to important beneficial effects on baroreflex function during severe orthostatic stress. It is interesting that the changes were observed in efferent sympathetic outflow and peripheral vascular tone and not in the HR response to LBNP. Whether this augmented vasomotor response is beneficial in terms of orthostatic tolerance remains to be determined. Preliminary data from Iwasaki et al. (29) suggest that orthostatic tolerance is not diminished by this magnitude of hypovolemia. Detailed examinations of baroreflex vascular control during hypovolemia versus deconditioning require further examination.

It is clear that the augmented vascular responses during hypovolemia were related to elevated sympathetic vasoconstrictor outflow. It may be that other circulating vasoactive hormones that increase in response to hypovolemia (20, 38, 53), such as angiotensin II (ANG II) and arginine vasopressin (AVP), may influence sympathetic reflex control. Although the levels and influence of such hormones were not addressed in the current study, we feel that such effects are unlikely because 1) low-pressure baroreflexes have little impact on vasopressin release (37) and 2) baroreflex control of AVP release requires periods of stimulation that appear to be longer than the 5-min bout of −40 mmHg LBNP used here (37). However, it may be that elevations in AVP associated with spironolactone-induced hypovolemia affected baroreflex sympathetic control as measured during LBNP. Previously, endogenous release of AVP influenced central sympathetic reflex responses primarily through effects on the area postrema and the nucleus tractus solitarius (26). However, the direction of this modulatory effect on baroreflex function appears to depend on whether hypotension or hypertension is the input stimulus. For example, AVP has been shown to augment the inhibitory effects of both cardiopulmonary (28) and arterial (18) baroreflexes. In contrast, AVP diminished the reflex increase in sympathetic outflow during hypotension (18, 26). Therefore, it is unlikely that elevated vasopressin levels influenced the augmented integrated baroreflex responses observed during hypovolemia in the present study.

Past research has demonstrated that elevated levels of ANG II can act within the central nervous system to stimulate sympathetic outflow (27, 34, 42) and may attenuate baroreflex inhibition of sympathetic nerve activity (49). However, other research has shown that ANG II either had no effect (24) or attenuated (43) baroreflex-mediated sympathetic responses to hypotension. Therefore, elevated levels of ANG II elicited by hypovolemia may partially explain the elevated sympathetic response at rest but are likely not involved in the elevated MSNA during LBNP. Regardless, the proportionate increases in baseline MSNA (23%), FVR (27%), and TPR (14%) during hypovolemia suggest that vasoconstrictor influences in addition to MSNA were minimal. Therefore, it is argued that the major factor determining the augmented TPR response was the concurrent increase in sympathetic discharge. However, the mechanisms mediating the altered baroreflex sympathetic function during integrated baroreflex unloading are uncertain. Evidence that sympathetic and vasomotor responses to postural stress after bed rest (48) or spaceflight (6) vary between individuals suggests that susceptibility to relative contributions of hypovolemia versus reflex sympathetic blood pressure control may produce important determinants of orthostatic tolerance after cardiovascular deconditioning.

In summary, the major finding of this study was that hypovolemia, without intervening bed rest or spaceflight effects on cardiovascular deconditioning, produced augmented sympathetic outflow at rest and during graded LBNP up to −40 mmHg. This resulted in greater systemic and peripheral vasoconstrictor responses. The combined analysis indicated that at lower levels of orthostatic stress (i.e., up to −20 mmHg LBNP), hypovolemia caused an upward shift in the MSNA and vasomotor versus CVP relationships without a change in the reflex gain. In contrast, the gain of the integrated baroreflex responses elicited by a greater degree of LBNP (i.e., −40 mmHg) was augmented in hypovolemia. The net result was an increase in MAP compared with baseline during hypovolemia at −40 mmHg LBNP compared with the hypotensive response observed in normovolemia. Additional studies are required to examine the effect of hypovolemia on orthostatic tolerance in the presence and absence of bed rest- or spaceflight-induced changes to baroreflex.
function. On the basis of current evidence, it may be proposed that hypovolemia in the absence of microgravity-induced cardiovascular deconditioning can provide a beneficial compensatory autonomic response to the impaired baroreflex vascular control that normally occurs in such situations.

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