Enhanced sympathoinhibitory response to volume expansion in conscious hindlimb-unloaded rats

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Mueller, Patrick J., and Eileen M. Hasser. Enhanced sympathoinhibitory response to volume expansion in conscious hindlimb-unloaded rats. J Appl Physiol 94: 1806–1812, 2003.—Prolonged exposure to microgravity or bed rest produces cardiovascular deconditioning, which is characterized by reductions in plasma volume, alterations in autonomic function, and a predisposition toward orthostatic intolerance. Although the precise mechanisms have not been fully elucidated, it is possible that augmented cardiopulmonary reflexes contribute to some of these effects. The purpose of the present study was to test the hypothesis that sympathoinhibitory responses to volume expansion are enhanced in the hindlimb-unloaded (HU) rat, a model of cardiovascular deconditioning. Mean arterial blood pressure, heart rate, and renal sympathetic nerve activity (RSNA) responses to isotonic volume expansion (0.9% saline iv, 15% of plasma volume over 5 min) were examined in conscious HU (14 days) and control animals. Volume expansion produced decreases in RSNA in both groups; however, this effect was significantly greater in HU rats (−46 ± 7 vs. −25 ± 4% in controls). Animals instrumented for central venous pressure (CVP) did not exhibit differences in CVP responses to volume expansion. These data suggest that enhanced cardiopulmonary reflexes may be involved in the maintenance of reduced plasma volume and contribute to attenuated baroreflex-mediated sympathoexcitation after spaceflight or bed rest.

Cardiovascular deconditioning; sympathetic nerve activity; cardiopulmonary receptor reflex; hindlimb suspension; orthostatic intolerance

METHODS

All procedures were performed according to the guidelines stated in the National Institutes of Health “Guide for the Care and Use of Laboratory Animals.” All protocols were approved by the University of Missouri-Columbia Animal Care and Use Committee.

HU. Male Sprague-Dawley rats (260–360 g, Harlan Sprague Dawley, Indianapolis, IN) were designated arbitrarily to either HU or control conditions. The HU method used was similar to that described previously (28). Briefly, HU rats were acclimated for a 3-day period to HU conditions by suspending them by their tails for short durations (1–3 h/day). On the fourth day, HU rats were anesthetized briefly (<10 min) with halothane (2%) to apply the tail harness and a thoracic cast (Schering-Plough Animal Health, Union, NJ). The tail harness consisted of a curved rigid support attached to the tail by moleskin and cloth tape. Hooks were fastened to the harness, and animals were suspended via a swivel appa-

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ratus such that the hindlimbs did not make contact with the cage floor. Thoracic casts were applied to prevent HU animals from reaching the tail apparatus and to reduce lordosis. Control animals were fitted with thoracic casts in a similar manner. HU animals were suspended at an angle of ~30–35° so that they were able to move freely about the cage where access to food and water was readily available. Control rats were returned to their home cages where they maintained normal cage activity. During HU conditions, animals were closely monitored for food and water intake, grooming, defecation, and urination. All animals remained under control or HU conditions for 14 days and were studied after removal from HU or control conditions. Specifically, these studies were performed while the rats were in the horizontal position to simulate a return from spaceflight or to normal upright posture after prolonged bed rest.

**Surgical procedures.** On the 12th day of the protocol animals were removed from their cages and instrumented for recordings of arterial blood pressure, heart rate (HR), and renal sympathetic nerve activity (RSNA). All surgical procedures were performed under halothane anesthesia (2%) with the rats intubated with a tracheal tube and secured in a rat restrainer (Plough Animal Health, Union, NJ), which was allowed to harden on insulating silver wires (0.005-in. diameter, 36 gauge; Medwire) threaded through Silastic tubing (0.025 in. ID). After isolation of a branch of the renal nerve, nerves and control catheters were exteriorized along with the electrode. Catheters were tunneled subcutaneously and exteriorized at the back of the neck. A ground wire was sewn into the muscle layer and exteriorized along with the electrode. Catheters were filled with heparinized saline (10 U/ml) and plugged with obturators. Animals were given subcutaneous fluids (20 ml saline) postoperatively. After recovery from anesthesia, HU rats were immediately returned to the suspension cage and control rats were returned to their home cages. All animals remained under control or HU conditions for an additional 2 days before experiments were performed.

Initial studies indicated that there was a trend for enhanced sympathetic nerve responses to volume expansion in HU animals. Because it was possible that these differences could be related to differences in stimulation of cardiopulmonary receptors, we instrumented additional animals (HU and control conditions) for measurements of central venous pressure (CVP) to estimate the level of cardiopulmonary receptor stimulation during the volume expansion protocol. CVP was measured via a catheter that was placed in the jugular vein and advanced to the level of the right atrium. Placement of the jugular catheter was verified at the end of each experiment by postmortem autopsy. In addition to the jugular catheter, these animals were also instrumented for measurements of arterial pressure and HR as described above.

**Experimental procedures.** After a 2-day recovery period, HU rats were removed from suspension cages and control animals were removed from their cages. Conscious rats were placed in an experimental cage with their own bedding and were studied ~1.5–3 h after acclimation to the experimental cage. As previously stated, these conditions were chosen specifically to simulate return from spaceflight or normal upright posture after prolonged bed rest. All experiments were conducted within a Faraday cage to reduce electrical noise. The arterial catheter (and jugular catheter when present) was connected to a pressure transducer to record pulsatile pressure. Mean arterial blood pressure (MAP) and mean CVP were derived electronically by using a low-pass filter. HR was determined from the pulsatile arterial blood pressure signal by a cardiometer.

RSNA was amplified 1,000 times by using a Grass preamplifier (P511) and filtered at 0.03 and 3 kHz by using high- and low-pass filters, respectively. Compound action potentials were monitored on a Tektronix 5113 storage oscilloscope and a Grass M8 audio monitor. The raw RSNA signal was rectified and integrated by using a root mean square (RMS) converter with a time constant of 28 ms. The rectified, integrated signal was then averaged and used as a relative measure of RSNA. At the end of the experiment, background noise was determined after ganglionic blockade [atropine (1 mg/kg) + hexamethonium (30 mg/kg) iv]. RSNA was determined by subtracting background noise from the average RSNA signal and setting the remaining control signal at 100%. Relative changes in RSNA were determined as percentage of this control.

**Experimental protocols.** Hemodynamic variables were measured during acclimation, and data were taken for at least 30–60 min before any experimental intervention to ensure stable MAP, HR, and RSNA levels. Once a stable baseline was obtained, control data were taken (~60 s), and volume expansion was performed by an intravenous infusion of isotonic saline (15% of calculated plasma volume over 5 min) on the basis of previously published estimates of plasma volume in rats (60 ml/kg) (5). Immediately after volume expansion, a second set of cardiovascular variables was taken during the first period of stable RSNA (typically within the first 30 s).

After determination of background noise, animals were euthanized (0.3 ml iv, Buthanasia-D Special, Schering-Plough Animal Health, Union, NJ). The soleus and plantaris muscles were removed from the right leg, blotted dry, and then weighed. Significant increases in hindlimb muscle weights and hindlimb muscle-to-body weight ratios served as verification of the effectiveness of the HU procedure (28, 31, 42).

**Data analysis.** Hemodynamic and RSNA data were obtained on a chart recorder (model 2107-8890-00, Gould, Cleveland, OH), written to paper, and analyzed by hand. Pulse pressure was determined by subtracting systolic pressure from diastolic pressure. Experiments were also taped on a videocassette recorder and, in the latter part of the study, obtained on a PowerLab data acquisition system. Because the system was utilized for only part of the study, it was used primarily to reproduce raw data figures. All data are presented as means ± SE. Hindlimb muscle weights and body weights were analyzed by Student’s t-test. Hemodynamic variables and sympathetic nerve responses were determined before (baseline) and immediately after volume expansion. Responses were examined by two-way ANOVA with group (control vs. HU) and treatment (baseline vs. volume expansion) as factors. When ANOVA indicated a significant interaction, differences between individual means were assessed by a least significant difference test (41). A value of P < 0.05 was deemed significant for all tests.

**RESULTS**

The effects of 14-day HU or control conditions on body weight, hindlimb muscle-to-body weight ratios, and resting hemodynamic variables are shown in Table 1. Similar to previous studies (31, 33, 34, 42), we verified the efficacy of our HU procedure by examining...
### Table 1. Effect of 14-day hindlimb-unloading or control conditions on body weight, hindlimb muscle-to-body weight ratios, and resting hemodynamic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 15)</th>
<th>Hindlimb Unloaded (n = 13)</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>311 ± 4</td>
<td>292 ± 7</td>
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<tr>
<td>Soleus muscle weight/body weight</td>
<td>0.442 ± 0.012 × 10^3</td>
<td>0.267 ± 0.069 × 10^3</td>
</tr>
<tr>
<td>Plantaris muscle weight/body weight</td>
<td>0.117 ± 0.016 × 10^3</td>
<td>0.101 ± 0.023 × 10^3</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>415 ± 8</td>
<td>442 ± 6^a</td>
</tr>
<tr>
<td>Resting MAP, mmHg</td>
<td>127 ± 2</td>
<td>132 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. HR, heart rate; MAP, mean arterial blood pressure. *P < 0.05 compared with control.

The extent of hindlimb muscle atrophy in HU rats. HU rats had a significantly lower soleus muscle-to-body weight ratio and a significantly lower plantaris muscle-to-body weight ratio, suggesting that significant atrophy did occur in these animals. Body weights on the day of experimentation appeared lower in HU rats and were nearly significantly compared with control animals (P = 0.06). Arterial blood pressure was not significantly different between groups, and, similar to previous studies involving individuals undergoing cardiovascular deconditioning (7, 10, 19), HU rats presented with a resting tachycardia compared with cage controls.

**Effect of HU on sympathoinhibitory responses to volume expansion.** Figure 1 represents typical results from one control (A) and one HU rat (B) exposed to isotonic volume expansion (0.9% NaCl iv, 15% of plasma volume over 5 min). This level of volume expansion resulted in no appreciable change in arterial pressure or HR in either the control or HU rat (Fig. 1). In contrast, volume expansion produced decreases in RSNA in both animals. Furthermore, similar to the group data, the decrease in RSNA was greater in the HU rat (50%) compared with the control rat (17%).

The average data from the volume expansion protocol are represented in Fig. 2. There were no significant effects of treatment (baseline vs. volume expansion) or group (HU vs. control) on MAP, indicating that MAP did not change in response to volume expansion in either group. Pulse pressure was slightly but significantly increased in response to volume expansion in both groups [control: change (Δ) 3 ± 1 mmHg; HU: Δ 2 ± 1.8 mmHg]. There was no effect of group and no interaction, indicating that the change in pulse pressure due to volume expansion was similar in control and HU rats. Volume expansion had no significant effect on HR in either group. However, there was a significant group effect for HR but no interaction. These data indicate that the resting tachycardia observed in the HU rats under control conditions was still evident after volume expansion and that HR was not altered by volume expansion in either group. With regard to RSNA responses, there was a significant decrease in response to volume expansion in both groups. Furthermore, because there was also a significant interaction between treatment and group, we tested for individual differences. The post hoc tests revealed that the decrease in RSNA in response to volume expansion was significantly greater in HU rats compared with control animals (−46 ± 7 vs. −25 ± 4%, respectively; P < 0.05).

**Effect of HU on CVP responses to volume expansion.** To examine whether volume expansion produced similar levels of activation of cardiopulmonary receptors in HU and control animals, we instrumented additional animals (control and HU) for measurement of CVP during volume expansion. Similar to the previous experiment, MAP did not change significantly with volume expansion (15% of plasma volume over 5 min) in control (Δ −2.3 ± 3.1 mmHg) or HU rats (Δ −1.6 ± 2.2 mmHg).
mmHg). There were small changes in HR after volume expansion (control: Δ7 ± 5 beats/min; HU: Δ6 ± 4 beats/min) that failed to reach statistical significance (P = 0.08). Figure 3 demonstrates the effects of volume expansion on CVP. There was a significant increase in CVP in both groups as indicated by an overall main effect of treatment. Importantly, there was no significant overall main effect of group and no interaction between treatment and group. These data suggest that CVP was not significantly different between control and HU rats at rest or after volume expansion.

DISCUSSION

The major finding of this study is that renal sympathoinhibitory responses to isotonic volume expansion are enhanced in HU rats. This augmented response occurred in HU rats despite similar increases in CVP. These data suggest that reflex neural regulation of arterial blood pressure and fluid balance is altered in individuals undergoing cardiovascular deconditioning. Furthermore, we speculate that this finding may provide a mechanism by which there is both diminished arterial baroreflex function and maintenance of an already reduced plasma volume in individuals that experience periods of cardiovascular deconditioning.

The simplest interpretation of an enhanced sympathoinhibitory response to volume loading is that cardiopulmonary reflexes are enhanced in HU rats. A number of previous studies have suggested that cardiovascular deconditioning enhances responses due to cardiopulmonary reflexes (2, 11–13). For example, Convertino et al. (11) reported that 7 days of 6° head-down bed rest produced an increase in the sensitivity of the CVP/forearm vascular resistance relationship when cardiopulmonary receptors were unloaded with lower body negative pressure. In addition, enhanced natriuresis and diuresis is exhibited by rats flown in space (44) and humans exposed to 7 days of head-down tilt bed rest (12). The present study extends these previous findings and suggests that these alterations are due at least in part to changes in sympathetic outflow vs. changes at the level of the end organ. It has also been suggested that cardiovascular deconditioning produces a shift in the “set point” of blood volume regulation (13). This hypothesis is based on findings that the gain of the cardiopulmonary reflex (based on measurements of CVP, urine volume rate, and renal sodium excretion) was similar after 48 h of head-down tilt but that the curve was shifted left and downward.

Fig. 2. Mean AP (MAP; A), heart rate (HR; B), and RSNA (C) responses to volume expansion (0.9% NaCl iv, 15% of plasma volume over 5 min) in control (○, n = 9) and hindlimb-unloaded rats (●, n = 10). Values are means ± SE. Neither group had significant changes in MAP or HR after volume expansion. Both groups demonstrated significant decreases in RSNA in response to volume expansion. The decrease in RSNA, however, was significantly greater in HU rats compared with controls. B: †significant main effect of group (i.e., HU vs. control), P < 0.05. C: *significant effect of treatment (i.e., volume expansion), P < 0.05 (by post hoc analysis); †significant effect of group, P < 0.05 (by post hoc analysis).

Fig. 3. Central venous pressure (CVP) responses to volume expansion (0.9% NaCl iv, 15% of plasma volume over 5 min) in control (○, n = 6) and HU rats (●, n = 6). Values are means ± SE. There were no significant differences in these responses between control and HU rats. * Significant main effect of treatment (i.e., volume expansion), P < 0.05.
It is possible that HU has no effect on the gain of the cardiopulmonary reflex and merely produces a shift in or along the reflex curve. The present study, in which cardiopulmonary receptors were only stimulated, does not allow us to evaluate this possibility. Further studies that examine unloading of cardiopulmonary receptors and can assess changes in baseline RSNA are required to test this hypothesis.

Enhanced sympathoinhibition to volume loading could contribute to several physiological consequences associated with cardiovascular deconditioning. Because a diminished plasma volume has been proposed to contribute to the predisposition toward orthostatic intolerance observed in some astronauts (22, 23), countermeasures such as isotonic fluid loading would be expected, in theory, to improve orthostatic tolerance. To the contrary, volume loading has been shown to be less than effective in offsetting orthostatic intolerance (6, 18). The present study suggests that one mechanism contributing to this effect may be an enhanced inhibition of RSNA during volume expansion, resulting in augmented cardiopulmonary reflex-mediated diuresis and natriuresis. Therefore, in our opinion, it will be important in subsequent studies to examine whether the observed alterations in renal nerve activity are related to changes in renal function and control of plasma volume.

Enhanced cardiopulmonary reflex function could also contribute to the consequences of cardiovascular deconditioning by influencing other autonomic reflexes. For example, previous studies have demonstrated that arterial baroreflex-mediated sympathoexcitation can be modulated in an inhibitory manner by activation of cardiopulmonary receptors (8, 25). Therefore, it is possible that an enhanced cardiopulmonary receptor reflex could contribute to diminished baroreflex-mediated sympathoexcitation in HU rats (28) and humans exposed to bed rest (20, 40). Further studies are necessary to determine whether this interaction occurs in HU rats and whether it contributes importantly to the reduction in baroreflex function produced by cardiovascular deconditioning.

There are several mechanisms by which cardiovascular deconditioning may produce an enhanced response to volume expansion. These include decreases in plasma volume (11, 21, 43), changes in afferent input or sensitivity, and alterations within the central nervous system pathways involved in the cardiopulmonary reflex (32). Although these are all distinct possibilities, we suggest it is likely to be some combination of some or all of these alterations. Data from our own laboratory suggest that central nervous system alterations in HU rats contribute to diminished arterial baroreflex function in the absence of changes in afferent sensitivity (19, 28–30). In addition, preliminary data suggest that alterations within the central nervous system may be involved in enhanced cardiopulmonary reflexes (32). Therefore, we speculate that alterations in synaptic transmission within brain areas involved in autonomic control are involved in the altered cardiovascular reflexes after cardiovascular deconditioning.

In contrast to the present results, the results of some studies suggest that responses to volume loading are unchanged (16, 17) or even attenuated (12, 27) after cardiovascular deconditioning. The reasons for this discrepancy are not clear, but it may be related to the duration of cardiovascular deconditioning. For example, brief periods of head-down tilt (24–72 h) are associated with attenuated responses (12, 27), and longer periods (6–10 days) are associated with normal (16, 17) or augmented responses (2). In addition, it has been reported that there is a 55% reduction in the urine output response to saline infusion during spaceflight (35). It is quite possible that during spaceflight or bed rest or after short-term exposure to deconditioning (12, 27), cardiopulmonary reflexes are attenuated, but after a return to Earth or normal upright posture from longer term deconditioning, this reflex is actually enhanced (Ref. 2, present study). Thus responses to volume expansion in deconditioned individuals are likely to depend on the type of deconditioning, the species and particular variable studied, and the duration of deconditioning (2).

The sympathoinhibitory response to volume expansion in conscious animals has been demonstrated in a number of species, including rats, rabbits, and cats (1, 15, 24, 38). Although one recent study in anesthetized rats suggests that the renal vasodilatory response to volume expansion is mediated primarily by arterial baroreceptors (9), the preponderance of evidence suggests that responses to volume expansion in conscious animals are mediated primarily by cardiopulmonary receptors (1, 14, 24, 37). In the present study, experiments were carried out in animals with intact arterial baroreceptors. Thus we cannot conclude with complete certainty that there was no involvement of the arterial baroreflex in the observed responses. Nonetheless, we suggest that the augmentation of the sympathoinhibition to volume expansion observed in HU rats is most likely due to differences in cardiopulmonary reflex function for the following reasons. As mentioned above, the majority of evidence suggests that responses to volume expansion are mediated primarily by cardiopulmonary receptors. In the present study, MAP was not altered by volume expansion, and the slight increase in pulse pressure was similar in control and HU rats. Finally, our laboratory has reported previously that the decrease in sympathetic nerve activity in response to activation of arterial baroreceptors is not altered appreciably by HU (28). Thus, even if arterial baroreceptors were activated by this protocol, the contribution of baroreceptors to sympathoinhibition would be expected to be similar in both groups. We propose that, taken together, the evidence suggests that the enhanced decrease in sympathetic nerve activity in response to volume expansion is likely due to an enhancement of cardiopulmonary receptor reflex function rather than enhanced arterial baroreceptor-mediated sympathoinhibition.

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There are some limitations to our study due to the fact that we recorded RSNA from conscious animals. First, we acknowledge that RSNA may or may not accurately reflect sympathetic outflow to other vascular beds, and thus our conclusions are limited as such. Second, we recorded compound action potentials from a multifiber preparation. In doing so, we cannot distinguish between efferent nerve fibers that contribute to renal function from those that are involved in control of renal blood flow. Lastly, we expressed RSNA in terms of percent change from control after normalizing all baseline RSNA data to 100%. Therefore, we did not determine whether resting RSNA was different between groups. Despite the above limitations, we believe the conclusions put forth are appropriate and valid.

In summary, 14 days of HU produces enhanced sympathoinhibitory responses to volume expansion in conscious rats despite similar increases in CVP. These data suggest that cardiovascular deconditioning alters cardiopulmonary reflex regulation of sympathetic nerve activity and body fluid balance possibly via central nervous system alterations. Furthermore, these findings may provide a mechanism for orthostatic intolerance observed after cardiovascular deconditioning. Specifically, an enhanced cardiopulmonary reflex may interact in an inhibitory manner with arterial baroreflex-mediated increases in sympathetic nerve activity and contribute to the maintenance of a decreased plasma volume via enhanced diuresis and natriuresis in response to fluid loading.

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