Hindlimb unloading alters nitric oxide and autonomic control of resting arterial pressure in conscious rats

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Mueller, Patrick J., C. Michael Foley, and Eileen M. Hasser. Hindlimb unloading alters nitric oxide and autonomic control of resting arterial pressure in conscious rats. Am J Physiol Regul Integr Comp Physiol 289: R140–R147, 2005. First published March 10, 2005; doi:10.1152/ajpregu.00820.2004.—After periods of microgravity or bed rest, individuals often exhibit reduced VO2max, hypovolemia, cardiac and vascular effects, and autonomic dysfunction. Recently, alterations in expression of vascular and central nervous system NO synthase (NOS) have been observed in hindlimb-unloaded (HU) rats, a model used to simulate physiological effects of microgravity or bed rest. We examined the effects of 14 days of hindlimb unloading on hemodynamic responses to systemic NOS inhibition in conscious control and HU rats. Because differences in NO and autonomic regulation might occur after hindlimb unloading, we also evaluated potential differences in resting autonomic tone and effects of NOS inhibition after autonomic blockade. Administration of nitro-L-arginine methyl ester (L-NAME; 20 mg/kg iv) increased mean arterial pressure (MAP) to similar levels in control and HU rats. However, the change in MAP in response to L-NAME was less in HU rats, that had an elevated baseline MAP. In separate experiments, atropine (1 mg/kg iv) increased heart rate (HR) in control but not HU rats. Subsequent administration of the ganglionic blocker hexamethonium (30 mg/kg iv) decreased MAP and HR to a greater extent in HU rats. Administration of L-NAME after autonomic blockade increased MAP in both groups to a greater extent compared with intact conditions. However, the pressor response to L-NAME was still reduced in HU rats. These data suggest that hindlimb unloading in rats reduces peripheral NO as well as cardiac parasympathetic tone. Along with elevations in sympathetic tone, these effects likely contribute to alterations in vascular control and changes in autonomic reflex function following spaceflight or bed rest.

The hindlimb-unloaded (HU) rat exhibits many of the same changes that occur in humans after microgravity or bed rest (22, 42). These include decreases in plasma volume (2, 15, 16, 34), diminished baroreflex function (41), indications of orthostatic intolerance (34), and reduced exercise capacity (14, 46, 64). Recently, alterations in the regulation of nitric oxide (NO) have been observed in this animal model and have been proposed to play a role in the cardiovascular adaptations to spaceflight or bed rest. For example, reductions in endothelial NO synthase (eNOS) occur in skeletal muscle arteries after hindlimb unloading (25, 55, 65). These changes have been proposed to contribute to diminished skeletal muscle vasodilation (13, 25, 35, 65) and decreased exercise capacity that occur after real or simulated microgravity (14, 30, 46, 64). In contrast, hindlimb unloading in rats also appears to increase the inducible form of nitric oxide synthase (iNOS) in large conduit vessels, including the carotid artery and aorta (32, 62). Increased NO produced by iNOS has been proposed to contribute to increases in vasodilation, decreases in vasoconstriction, and orthostatic intolerance (51, 69). Finally, along with these peripheral effects, increases in NOS within the central nervous system have been reported following hindlimb unloading (43, 62) where NO may influence autonomic regulation (27, 48, 67). Therefore, alterations in both peripheral and central NO systems appear to accompany cardiovascular deconditioning and may impact cardiovascular regulation significantly.

Evidence to date also suggests that exposure to microgravity or bed rest is associated with alterations in autonomic tone. For example, resting tachycardia is often observed after spaceflight, bed rest, and hindlimb unloading (3, 19, 22, 53). It is possible that resting tachycardia could be due to reduced cardiac parasympathetic tone, increased cardiac sympathetic tone, or both. Indeed, one study using heart rate variability has reported both reduced parasympathetic tone and increased sympathetic tone to the heart in astronauts postflight (40). However, to our knowledge, more direct assessments of cardiac parasympathetic and sympathetic tone are lacking in studies of real or simulated microgravity. Changes in autonomic tone, along with alterations in NO regulation, could contribute to alterations in vascular control and changes in autonomic reflex function following spaceflight or bed rest.

The purpose of the present study was to evaluate the role of NO and the autonomic nervous system in the maintenance of arterial pressure after hindlimb unloading in rats. We hypothesized that hindlimb unloading reduces overall NO-mediated dilation and resting parasympathetic tone to the heart. We also hypothesized that the contribution of the sympathetic nervous system is decreased in HU rats, a model used to simulate physiological effects of microgravity or bed rest.
system to arterial pressure is increased by hindlimb unloading. To test these hypotheses, we examined hemodynamic responses to generalized blockade of NO by systemic NOS inhibition in control and HU rats. Responses to NOS inhibition are due to removal of vascular and possibly central effects of endogenous NO. Because differences in autonomic regulation exist after hindlimb unloading, these experiments also evaluated resting autonomic tone in control and HU rats. Finally, to remove the influences of reflex compensations or centrally produced NO on autonomic outflow (27, 48, 67), we also examined responses to NOS inhibition under conditions of ganglionic blockade.

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 reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee. Food (FormulaLab Diet, 0.28% sodium; Purina, St. Louis, MO) and water were available ad libitum to both groups of animals.

Hindlimb unloading. Male Sprague-Dawley rats (275–330 g; Harlan Sprague Dawley, Indianapolis, IN) were subjected to HU or control conditions for 14 days according to methods described previously (41, 42, 44). Briefly, rats were acclimated to HU conditions for a 3-day period by tail suspension for short durations (1–3 h/day) with removable strips of cloth tape. On the fourth day, rats designated for hindlimb unloading were anesthetized with Halothane (2%) briefly (<10 min) to allow application of a thoracic cast (Schering-Plough Animal Health, Union, NJ) and a tail harness. The tail harness was a curved, rigid support attached to the tail with cloth tape and moleskin. Casts were applied to the thorax to reduce lordosis and to prevent HU animals from reaching the tail apparatus. The tail harness was connected to a swivel apparatus that allowed the animals ready access to food and water and the ability to move about the cage freely without their hindlimbs making contact with the cage floor. Animals were suspended at an angle of ~30–35°, similar to our previous studies (41, 44) and as recommended by others (42). Control animals were fitted similarly with thoracic casts and were returned to their home cages, where they maintained normal cage activity. Animals were closely monitored during HU conditions for overt signs of stress by daily examination of general behaviors including food and water intake, grooming, defecation, and urination. Animals exhibiting overt signs of stress or excessive weight loss (>10%) were excluded from further study similar to our previous studies (41, 44) and as recommended by others (42).

Surgical procedures. After 12 days of HU or control conditions, animals were prepared for conscious recordings of mean arterial pressure (MAP) and heart rate (HR). Surgical procedures were performed using aseptic techniques. Under Halothane anesthesia (2%), catheters (PE50 fused to PE10) were placed in the left femoral artery and vein for measurement of arterial pressure and drug administration, respectively. Catheters were tunneled subcutaneously, exteriorized at the infrascapular region, and filled with heparinized (10 U/ml) saline. Catheters were then plugged, and animals were given subcutaneous fluids (10 ml saline). Animals were allowed to regain consciousness and were then immediately returned to HU or control conditions to minimize significant weight bearing by the hindlimbs in HU rats. The animals remained under control or HU conditions for an additional 2 days before experimentation.

Experimental procedures. After the 2-day recovery period from surgery, conscious control and HU rats were removed from their cages and weighed, and tail harnesses (on HU rats) were removed. Animals were then placed in an experimental cage that contained the bedding from their home cage. All experiments were performed in the same isolated, quiet room to minimize external influences on hemodynamic measurements. Both groups of animals were studied in the horizontal position (i.e., all limbs weight bearing) to simulate a return from spaceflight or to normal upright posture after prolonged bed rest. Experiments were conducted between 0.5 and 3 h after removal from control or HU conditions. The femoral arterial catheter was connected to a pressure transducer positioned at the level of the heart to record pulsatile arterial pressure. MAP was derived electronically using a low-pass filter, and HR was determined from the pulsatile arterial pressure signal by a cardiococheteter.

Experimental design. Hemodynamic variables were monitored during acclimation, and data were recorded for at least 30–60 min before any experimental intervention to ensure stable MAP and HR. Blood pressure was determined to be stable if there were no obvious trends for increases or decreases for at least 10 min. This block of 10 min preceding injections was taken as control data. Various blockers were then administered and peak responses were recorded. At the end of each protocol, animals were euthanized (0.3 ml iv, Beuthanasia-D Special; Schering-Plough Animal Health, Union, NJ), and the soleus and plantaris muscles were removed from the right leg, blotted dry, and then weighed. Along with the observation of resting tachycardia in HU animals (36, 41, 44), significant decreases in these hindlimb postural muscle weights (i.e., soleus and plantaris) and muscle weights relative to final body weight served as verification of the effectiveness of the hindlimb unloading procedure as described previously (41, 42, 44, 60).

Experimental protocols. In the first set of experiments, the overall contribution of endogenous NO to resting arterial pressure was examined in control and HU rats. Nitro-l-arginine methyl ester (l-NAME; 20 mg/kg; Sigma Chemical, St. Louis, MO) was administered as an intravenous bolus, and the peak blood pressure response was recorded (typically within 10 min). This dose of l-NAME was based on previous doses used in conscious rats that produced a peak blood pressure response within 10 min (20, 23). In a separate group of animals, we initially evaluated autonomic function. First, methyl atropine (1 mg/kg iv; Sigma Chemical) was administered to block parasympathetic influences and to determine resting parasympathetic tone in control and HU animals. Subsequently, hexamethonium (30 mg/kg iv; Sigma Chemical) was administered to produce complete ganglionic block (44). Because hexamethonium was administered in the presence of methyl atropine, the acute response to hexamethonium also served to determine the level of remaining sympathetic tone in control and HU animals. Because pressor responses to NO are likely buffered by arterial baroreflexes and because NO has both peripheral effects and central effects on the sympathetic nervous system, we tested the contribution of NO to resting arterial pressure in the presence of autonomic blockade. Five minutes after the administration of hexamethonium, l-NAME (20 mg/kg iv) was administered and peak MAP responses were again recorded along with simultaneous HR values.

Data analysis. Hemodynamic data were obtained on a chart recorder (model 2107-8890-00; Gould, Cleveland, OH), written to paper, and analyzed by hand. All data are presented as mean ± SE. Hindlimb muscle weights, hindlimb muscle weights relative to final body weight, and overall resting hemodynamic variables were analyzed using Student’s t-test for independent groups. Body weights were examined using two-way analysis of variance (ANOVA) with group (control vs. HU) and time (days 1 and 14) as factors. Individual protocols of hemodynamic variables were determined before (baseline) and at the peak change in MAP after each intervention. Absolute values and absolute changes from the appropriate baseline were examined using two-way ANOVA with group (control vs. HU) and condition (baseline vs. drug) as factors. When ANOVA indicated a significant interaction, differences between individual means were assessed using a post hoc Tukey’s test. Statistical analyses were performed utilizing a commercially available software package (SigmaStat; SPSS, Chicago, IL). A P value <0.05 was deemed significant for all tests.
autoinhibitory blockade was not different compared with the original resting values (Fig. 3).

Effect of hindlimb unloading on response to NOS inhibition after ganglionic blockade. Five minutes were allowed between the administration of hexamethonium and L-NAME for hemodynamic parameters to stabilize. Before the administration of L-NAME, absolute MAP was significantly higher than the peak decrease produced by hexamethonium (Fig. 3). At this time point, MAP was still significantly lower than the initial control level, and neither MAP nor HR was significantly different between groups (Fig. 3). Subsequent administration of L-NAME produced an increase in MAP in control and HU rats. The pressor response after total autonomic blockade was significantly greater than that observed in the intact state (Fig. 2). Peak MAP, however, was significantly lower in HU rats compared with controls after administration of L-NAME in the presence of autonomic blockade (Fig. 3). Thus the absolute change in MAP in HU rats was significantly less than in control rats (Fig. 2, right). The attenuated response to L-NAME was observed in HU rats whether expressed relative to the baseline just before L-NAME administration (Fig. 2) or relative to the level of MAP at the peak depressor response to hexamethonium (control ∆MAP, 99 ± 5 mmHg; HU ∆MAP 87 ± 6 mmHg; P < 0.05). Despite the large increase in MAP, L-NAME produced no significant changes in HR after application of atropine and hexamethonium (Fig. 3), consistent with effective ganglionic blockade in both groups.

RESULTS

Effects of hindlimb unloading. Table 1 contains pooled data from all rats in this study and includes body weights and absolute and relative wet weights of hindlimb muscles in control animals and animals that were hindlimb unloaded for 14 days. Control and HU rats had similar body weights before control or HU conditions were imposed. On the day of the experiment (day 14), HU rats had significantly lower body weights than cage controls. HU rats exhibited signs of hindlimb muscle atrophy (42, 45, 60) as evidenced by significantly lower absolute wet weight and wet weight relative to body weight (Table 1).

Effect of hindlimb unloading on response to NOS inhibition alone. Baseline MAP and HR values were significantly higher in HU animals (Fig. 1). Administration of L-NAME (20 mg/kg iv) increased MAP in both groups. Absolute peak blood pressures after administration of L-NAME were not significantly different between groups (Fig. 1). However, the change in MAP was attenuated in HU rats (Fig. 2A, left). In contrast, L-NAME produced equivalent decreases in HR in HU and control rats (Figs. 1 and 2B, left) such that the resting tachycardia in HU animals remained after L-NAME (Fig. 1).

Effect of hindlimb unloading on response to autonomic blockade. Baseline resting MAP and HR values were significantly higher in HU animals (Fig. 3). To block parasympathetic activity and determine resting cardiac parasympathetic tone in control and HU animals, we initially administered methyl atropine (1 mg/kg iv). Methyl atropine did not significantly alter MAP in control or HU rats (Fig. 3). In contrast, methyl atropine produced a significant increase in HR only in control rats, resulting in a significantly greater change in HR compared with HU rats (Figs. 3 and 4). HR was still significantly higher in HU rats after methyl atropine (Fig. 3). Subsequent administration of hexamethonium (30 mg/kg iv) reduced MAP in HU and control rats to a similar level (Fig. 3). However, the decrease in pressure was greater in HU compared with control rats (Figs. 3 and 4). Application of hexamethonium after atropine produced significant decreases in HR in both groups, and the absolute decrease in HR was greater in HU compared with control rats (Fig. 4). HU rats maintained a significantly higher HR after complete autonomic blockade (Fig. 3). In HU rats, absolute HR after the combination of atropine and hexamethonium was significantly lower than the original resting value (Fig. 3). In contrast, in control animals, absolute HR after
DISCUSSION

The major findings of this study are that responses to systemic inhibition of NOS are blunted by 14 days of hindlimb unloading in rats. These responses were attenuated both under control conditions and in the absence of the influence of the autonomic nervous system. These data suggest that the overall effects of peripheral NO are reduced by simulated microgravity or bed rest. HU rats also had reduced HR responses to atropine, but the decreases in HR and MAP to hexamethonium were enhanced. These data suggest that simulated microgravity or bed rest reduces parasympathetic tone to the heart and enhances sympathetic tone to the heart and possibly the vasculature.

The simplest interpretation of reduced responses to systemic NOS inhibition is that overall NO-mediated dilation is reduced by hindlimb unloading in rats. These responses were attenuated both under control conditions and in the absence of the influence of the autonomic nervous system. These data suggest that the overall effects of peripheral NO are reduced by simulated microgravity or bed rest. HU rats also had reduced HR responses to atropine, but the decreases in HR and MAP to hexamethonium were enhanced. These data suggest that simulated microgravity or bed rest reduces parasympathetic tone to the heart and enhances sympathetic tone to the heart and possibly the vasculature.

Fig. 2. Peak change (Δ) in MAP (A) and HR (B) in response to systemic inhibition of NOS with L-NAME (20 mg/kg iv) under intact conditions or after ganglionic blockade with hexamethonium (30 mg/kg iv) and atropine (1 mg/kg iv) in control (n = 8 for both intact and ganglionic blockade) and HU rats (n = 7 for intact, n = 6 for ganglionic blockade). Ganglionic blockade augmented the pressor response and reduced the bradycardic response to L-NAME in both control and HU rats. The pressor response to L-NAME was blunted by hindlimb unloading under both intact and ganglionic blockade conditions. The bradycardic response to L-NAME was unaffected by hindlimb unloading under either condition. * P < 0.05, effect of ganglionic blockade. #P < 0.05, HU vs. CC.

Fig. 3. MAP and HR during sequential blockade of parasympathetic tone with atropine (Atrop, 1 mg/kg), ganglionic blockade [hexamethonium (Hex; 30 mg/kg)], and systemic NOS (20 mg/kg L-NAME) in control (n = 8) and HU rats (n = 6). MAP and HR were significantly higher in HU rats under control conditions (baseline 1) and after atropine. Hexamethonium decreased MAP in both groups to similar levels. After L-NAME, MAP increased in both groups, but peak MAP was lower in HU rats. HR increased after atropine only in controls (baseline 1 vs. Atrop). Hexamethonium decreased HR significantly in both groups. However, HR was higher in HU rats after hexamethonium. Post hoc analysis also indicated that HR was significantly reduced by Hex to a level that was below initial baseline values in HU but not in control rats (†P < 0.05, Hex vs. baseline 1). HR was unchanged in both groups by L-NAME. *P < 0.05, compared with value in previous condition. #P < 0.05, HU vs. CC.

Fig. 4. Peak change in MAP and HR in response to sequential blockade of parasympathetic tone (Atrop) and ganglionic blockade (Hex) in control (n = 8) and HU rats (n = 6). MAP effects of atropine were similar between groups. The depressor response to hexamethonium was enhanced by HU. The tachycardic effect of atropine was reduced by hindlimb unloading, whereas the bradycardic effect of hexamethonium was enhanced in HU rats. #P < 0.05, HU vs. CC.
spaceflight (30). To our knowledge, the present study provides the first evidence in conscious animals that reduced NO following hindlimb unloading has consequences in controlling resting arterial blood pressure.

Two possible mechanisms by which hindlimb unloading may reduce NO-mediated dilation are via a reduction in the bioavailability of NO or a reduction in the responsiveness of the vascular smooth muscle to NO. With regard to the bioavailability of NO, hindlimb unloading has been shown to reduce eNOS expression in skeletal muscle and mesenteric arteries, likely decreasing NO production (25, 32, 55, 65). In addition, hindlimb unloading also has been shown to increase oxidative stress in skeletal muscle (28), which could reduce NO bioavailability. With regard to the responsiveness of the vascular smooth muscle, reduced sensitivity to the NO donor nitroprusside has been reported after hindlimb unloading (12). Other studies have reported a reduction in endothelium-dependent dilation in the absence of a change or an increase in endothelium-independent dilation (25, 33, 55, 71). Finally, structural changes in the vasculature that have been observed after hindlimb unloading (6, 70, 71) may play a role in altered vascular function, although not all studies agree with this possibility (47). Therefore, reductions in the bioavailability of NO and the responsiveness of the smooth muscle vasculature to NO may independently or collectively reduce NO-mediated dilation after simulated microgravity or bed rest.

The stimulus by which real or simulated microgravity or bed rest may reduce NO-mediated dilation is speculative. Reports of decreased calf blood flow in astronauts (63), reduced forearm vascular conductance in bed rest patients (11), and reduced hindlimb blood flow in HU rats (7, 13, 36, 52) suggest that shear stress may be reduced by these stimuli. McCurdy et al. (35) have argued that decreases in shear stress may account for decreases in eNOS and reductions in endothelium-mediated dilation after hindlimb unloading (12, 13, 25, 32, 55, 65). Therefore, the mechanisms by which real or simulated microgravity or bed rest affects NO expression and endothelial function may involve reductions in shear stress, but further studies are required to examine this issue.

In contrast to the attenuated responses to systemic NOS inhibition observed in the current study, there is also evidence for an upregulation of NOS after hindlimb unloading. Increases in NO in specific vessels of HU rats have been proposed to contribute to increased dilation and decreased constriction observed in isolated segments from these vessels (51, 54) and to enhanced pressor responses to iNOS inhibitors in anesthetized animals that have undergone hindlimb unloading (54, 62). Zhang and colleagues (69, 72) have proposed that hindlimb unloading produces opposite effects on vascular regulation in regions above and below the heart because of opposite effects of hindlimb unloading on transmural pressure and possibly shear stress in these vessels. These effects could be mediated by regionally selective regulation of NOS as discussed by Ma et al. (32). In addition, there may be differential effects of hindlimb unloading on the different isoforms of NOS in various vascular beds. Differences in the response to systemic NOS inhibition after hindlimb unloading in the present study would be expected to be, in part, a summation of responses from vascular beds that exhibit increased NOS expression and responses from vascular beds that exhibit decreased NOS expression. Our data suggest that overall systemic NO-mediated dilation is reduced in conscious rats after hindlimb unloading.

Previous studies suggest that NOS in the brain is increased after hindlimb unloading (43, 62). In general, it is believed that the actions of NO in the brain are primarily sympathoinhibitory, because blockers of NOS produce elevations in blood pressure and sympathetic nerve activity when microinjected into particular cardiovascular brain regions (5, 59, 68). The fact that we observed a reduced pressor response to l-NAME under control conditions suggests that the peripheral downregulation of NOS or decreases in NO vascular sensitivity, or both, contribute more to the cardiovascular response to systemic l-NAME than central upregulation of NOS. It is possible that the short time course during which we examined NOS inhibition (10 min) did not allow for full blockade of NOS in the brain, because a longer time course appears to be required for central NOS inhibition after systemic administration of NO inhibitors (24, 37, 61). Clearly, further studies are required to assess the actions of increased neural NO and its role in cardiovascular alterations produced by hindlimb unloading (43, 62).

Several studies including the present one have reported a resting tachycardia after spaceflight, bed rest, and hindlimb unloading (1, 3, 8, 21, 41, 44, 56, 58). In the present study, tachycardic responses to atropine were diminished whereas bradycardic responses to subsequent administration of hexamethonium were enhanced in HU rats. These data suggest that parasympathetic tone to the heart is reduced by hindlimb unloading and that sympathetic tone to the heart is augmented. These findings are supported by previous studies in astronauts postflight who exhibit evidence of reductions in parasympathetic and enhanced sympathetic tone to the heart (40). These alterations in autonomic tone are likely to contribute to the resting tachycardia observed after spaceflight, bed rest, and hindlimb unloading (1, 8, 21, 41, 44, 56, 58). In addition, it is possible that there are differences in intrinsic HR, because in the present study absolute HR values were higher in HU compared with control rats after complete ganglionic blockade.

**Experimental limitations.** We recognize that the present experiments involved some limitations. We only recorded arterial pressure and HR responses to NOS inhibition. Thus we are limited in our conclusions regarding potential differences in the effects of NOS inhibition on peripheral resistance, cardiac output, and ventricular performance. Furthermore, it is possible that hindlimb unloading has no effect on peripheral NO-mediated dilation under normal conditions and that the difference in the pressor response to l-NAME alone is due to differences in resting arterial pressure, changes in reflex compensation, or alterations in central NO. Although we cannot eliminate these possibilities entirely, we believe them to be unlikely for the following reasons. 1) Differences in the pressor response to l-NAME still existed after ganglionic blockade, when MAP values were similar. These data suggest that reduced pressor responses in HU animals under control conditions are not solely due to differences in resting MAP. 2) Because pressor responses to l-NAME were still attenuated in HU rats after ganglionic blockade, alterations in reflex compensation or in the influence of central NO could not account for these differences. 3) Although the difference in the pressor response to l-NAME between HU and control animals appeared to be smaller after ganglionic blockade, our statistical
analysis did not reveal a significant difference (i.e., no interaction). These data further support the theory that the difference in the pressor response between the two groups appears to be of vascular origin rather than a difference in central NO or reflex compensation. With regard to the latter, our conclusions are also substantiated by a previous report in which baroreflex-mediated sympathoinhibition and bradycardia to increases in arterial pressure were relatively unchanged by 14 days of hindlimb unloading (41). However, we did not record sympathetic nerve activity in these animals, and other factors could influence HR responses. Although further studies are required to fully evaluate whether differences in reflex compensation or central actions of NO occur in response to systemic NO inhibition, our results support the main conclusion of the present study that differences in vascular NO appear to contribute to differences in arterial pressure regulation following hindlimb unloading.

HU rats had higher resting MAP compared with control animals. This difference in MAP was normalized between groups by ganglionic blockade. In addition, hexamethonium produced a greater reduction in HR in HU animals. These data suggest that hindlimb unloading increases sympathetic tone to the heart and possibly the vasculature. Along with reductions in basal NO-mediated dilation, these differences likely contribute to the higher resting mean arterial blood pressures in the present study.

In summary, our data suggest that 14 days of hindlimb unloading produces reductions in basal NO-mediated dilation. In addition, parasympathetic tone to the heart appears to be decreased, whereas sympathetic tone to the heart and possibly the vasculature may be increased. The reductions in NO-mediated dilation may provide a mechanism for reduced exercise tolerance and for orthostatic intolerance observed after cardiovascular deconditioning. Specifically, diminished NO-mediated dilation may lead to reduced skeletal muscle vasodilation during exercise and reduced cerebral blood flow during orthostatic challenges. Alternatively, reduced NO may also serve to aid in orthostasis by maintaining a higher level of arterial pressure during an orthostatic challenge. Finally, increased baseline arterial pressure along with resting tachycardia also appears to reflect alterations in autonomic tone. These changes in resting autonomic function produced by hindlimb unloading may be related to or indicative of alterations in reflex autonomic function as observed previously by our laboratory (41, 44).

Perspectives

Although some previous studies have reported that microgravity produces small but significant elevations in resting arterial pressure in humans (3, 4), the majority of studies report little or no change in humans after real or simulated microgravity (18, 19, 39, 49, 50). Interestingly, the range of arterial pressures in our control and HU rats appears to fall in the range of conscious normotensive rats. Therefore, the difference in resting arterial pressure between control and HU rats in the present study appears to differ at the very least in magnitude compared with the majority of human studies. One possible explanation for the higher resting arterial pressure in our HU rats could be the extent to which NO is reduced and sympathetic nervous system activity is elevated in our study compared with others. Alternatively, because multiple mechanisms are involved in the regulation of resting arterial pressure, similar alterations in NO and sympathetic tone may occur in humans but may be compensated for by other mechanisms that regulate arterial pressure. In this regard, there are several studies in humans that have reported increases in measures of sympathetic nervous system activity (26, 31, 40, 58, 66) after real or simulated microgravity in the absence of a significant increase in resting arterial pressure (31, 58). These data highlight the complexities in trying to understand the mechanisms by which microgravity alters blood pressure regulation. Finally, we cannot eliminate the possibility that alterations in NO and sympathetic activity differ between real and simulated microgravity as well as animal models of microgravity. Because there were no differences in experimental conditions between control and HU rats in the present study, the increase in resting blood pressure appears to be due to the HU condition itself. The reasons for the differences in resting blood pressures observed between studies are not clear but are likely influenced by a number of variables including the duration of deconditioning (38), the time point and body positioning at which variables are measured after deconditioning (38), the influence of countermeasures (4), and whether individual subjects are classified as presyncopal or nonpresyncopal (38). Because we cannot distinguish between these possibilities in the current study, further studies in both animals and humans appear to be required to resolve this issue.

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