Hindlimb unloading elicits anhedonia and sympathovagal imbalance

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HUMANS EXPOSED TO PROLONGED bed rest or microgravity experience deconditioning of the cardiovascular system due to widespread central and peripheral physiological adaptations. On return to an upright posture in Earth’s gravitational field, these individuals display a number of adverse effects including reductions in plasma volume and exercise capacity, resting tachycardia, and orthostatic intolerance (4, 6, 9, 69).

Previous studies have indicated that many of these adverse consequences can be attributed to attenuated autonomic reflexes in both humans (6, 31, 62) and animal models that simulate bed rest and microgravity (24, 30, 41, 48, 49). Specifically, these effects include attenuated baroreflex function in both rodents and humans (9, 12, 30, 41) as well as changes in heart rate variability (HRV) following spaceflight and bed rest in humans (3, 39, 61, 63). In addition, changes within the central nervous system component of these reflexes has been shown to play a significant role in these attenuated reflex responses (24, 43, 45). These findings have lead to the generalized hypothesis that cardiovascular deconditioning is associated with autonomic nervous system dysfunction both in terms of relative contributions between the sympathetic and parasympathetic limbs of the autonomic nervous system (sympathovagal balance) and attenuated homeostatic reflexes due to changes within the central nervous system.

In addition to autonomic deficits, psychological depression has also been reported in astronauts during spaceflight (32, 33) as well as in healthy individuals confined to both horizontal (26, 66) and head-down tilt bed rest (25, 27, 66). Although these effects in humans may be confounded by psychosocial and psychosomatic effects, findings indicate that psychiatric stability can be altered through the course of cardiovascular deconditioning. While little insight exists as to the mechanisms responsible for these effects following cardiovascular deconditioning, the high incidence of comorbidity of psychological depression and cardiovascular dysfunction is well established in both human (2, 7, 14, 54, 58, 59, 71) and animal models (15, 17, 20–22, 42; for recent reviews see Refs. 18, 19).

Anhedonia, the reduced responsiveness to pleasurable stimuli, is a predominant feature of psychological depression in humans and rats (1, 34, 53, 72, 73). Our laboratory has recently reported in a rat model of psychological depression that anhedonia was associated with disordered sympathovagal tone (16, 20). Despite the well-established coexistence of reduced autonomic function and psychological depression among a wide range of patient populations and animal models, to our knowledge there have been no studies in which systematic examination of both psychological and physiological effects have been investigated in cardiovascular deconditioned animals or humans.

Although it is generally accepted that depression leads to physical inactivity, the idea that extreme physical inactivity and cardiovascular deconditioning produced through bed rest or microgravity leads to depression is less well known. In humans, the prevalence appears to be greater among bed rest studies than microgravity studies, most likely due to the strict psychological screening and selection criteria among astronauts (33). Even so, disorders of mood and thought have been reported among astronauts with an increased incidence in longer duration missions that may be confounded by psychosocial factors (32, 33). Head-down tilt bed rest in healthy, young men has been found to be associated with enhanced depressive and neurotic levels (25, 27). In another study, it was found that head-down tilt with balanced traction was associated with...
with a larger increase in depression scores than following horizontal bed rest, although there was a greater incidence of back pain in the head-down tilt group (66). Thus, although the incidence of psychological depression appears to be increased following cardiovascular deconditioning in humans, results in humans may be confounded by other variables, including psychosocial and psychosomatic factors. In addition, none of these studies measured cardiovascular parameters or attempted to characterize the relationship between psychological measures with cardiovascular function.

The hindlimb-unloaded (HU) rat is a well-established ground-based animal model used to study mechanisms responsible for cardiovascular deconditioning following bed rest and spaceflight (for recent review see Ref. 46). The physiological effects of HU in rodents are similar to those experienced by humans following cardiovascular deconditioning. These effects include an initial central shift in fluids, diuresis, natriuresis, and reduced plasma volume and blood volume (37, 60, 67). In addition, on removal from hindlimb unloading, rats exhibit typical signs of deconditioning: resting tachycardia, reduced exercise capacity, and effects consistent with orthostatic intolerance (37, 41, 56, 74). Previous data from our laboratory and others indicate that HU in rodents results in attenuated autonomic reflexes (12, 24, 30, 41, 49) and specifically that attenuated baroreflex control of sympathetic nerve activity is normalized through short-term administration of pharmacological antidepressant treatment (44). Thus the HU rat model of cardiovascular deconditioning provides an excellent model for controlled study of both anhedonia and cardiovascular autonomic function.

We hypothesized that the HU rats would exhibit both symptoms of anhedonia and sympathovagal imbalance. To test this hypothesis, we used measures of sucrose preference and intracranial self-stimulation (ICSS) to assess anhedonia during and after HU. In addition, autonomic blockade, HRV, and spectral analysis of blood pressure were used to determine relative cardiac sympathetic and parasympathetic tone and autonomic control of blood pressure, respectively, following HU.

**METHODS**

**General Experimental Design**

Two sets of experiments were performed to test the central hypothesis that HU would elicit both anhedonia and attenuated autonomic function. In experiment 1, ICSS was assessed at baseline, immediately following, and after recovery from the HU or casted control (CC) condition. Rats (n = 12) were implanted with ICSS electrodes and randomly assigned to HU and CC groups with baseline ICSS values obtained before HU or CC instrumentation. Animals were confined to the HU (n = 6) or CC (n = 6) condition for a period of 14 days. ICSS responses were obtained in all animals in the normal posture within 2 h following removal from HU or CC condition. ICSS responses were again obtained following a 7-day period of recovery during which time all animals were maintained in a normal cage environment in all HU and CC animals. Following the end of the experiments rats were killed, and soleus and plantaris muscle wet weights were obtained to determine the extent of recovery from the HU procedure.

In experiment 2, rats (n = 24) were randomly assigned to the CC (n = 12) or HU (n = 12) group and then adapted to sucrose and the HU procedure. Sucrose preference was measured in CC (n = 12) and HU (n = 12) animals at baseline (before CC or HU instrumentation in the normal posture) and on day 11 of the 14-day HU or CC protocol. Animals were maintained in the CC or HU posture while sucrose preference was measured on day 11. On day 12, animals were surgically instrumented with femoral arterial and venous catheters for measurement of arterial pressure and administration of pharmacological autonomic blockers, respectively. Following immediate recovery from anesthesia, animals were returned to the CC or HU condition for 48 h of additional recovery. On the 14th day of the protocol, animals were removed from cages, allowed to stand in the normal posture, and following a 1-h acclimation period, baseline arterial blood pressure was collected over a period of 1 h followed by autonomic blockade. Following the end of experiments between the hours of 1500 and 1700, animals were decapitated (unanesthetized), and trunk blood was collected for measurement of plasma corticosterone concentration. Plantaris and soleus muscle wet weights were recorded for verification of the HU procedure.

**Subjects**

Male, Sprague-Dawley rats (250–350 g) were used for the experimental procedures. Animals were housed individually. Food (Purina rat chow 5012) and water were available ad libitum for the duration of the experiments with the exception of the 20 h before sucrose preference testing. Temperature was maintained at 22 ± 2°C, and the light cycle was held at 12:12 with lights on at 0600 h. Rats were allowed 1 wk to acclimate to the surroundings before any experimentation began. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and they were approved by the University of Iowa Institutional Animal Care and Use Committee.

**HU Procedure**

HU was induced through elevation of the hindlimbs with a harness attached to the proximal two-thirds of the tail by techniques previously described (41). Briefly, two hooks were attached to the tail with moleskin adhesive material. A curved rigid support made of lightweight plastic (X-lite splint, AOAKirschner Medical, Timonium, MD) was placed beneath the tail to allow adequate blood flow. The hooks were connected by a wire to a swivel apparatus at the top of the cage, and the hindlimbs were elevated so there was no contact with supportive surfaces. Rats were maintained in a suspension angle of ~30–35°. A small thoracic cast made from plaster of Paris was applied to reduce lordosis and help prevent the rats from reaching the tail apparatus. CC rats had thoracic casts applied but were maintained in a normal cage environment. Animals were adapted to the HU cage apparatus by temporarily being suspended with a piece of athletic tape attached to the tail for 1–2 h, 2–3 days prior to full instrumentation. Animals remained in the HU or CC conditions for a total of 14 days. Body weights were recorded before and after the control or hindlimb-unloading period. During the unloading protocol, the rats were closely monitored several times daily for adequate food and water intake, grooming behavior, and urination and defecation. Body weight was monitored on the seventh day of the HU protocol with the animal maintained in the unloaded position to ensure that animals were not experiencing excessive loss of body weight. Identical procedures were used for eliciting HU in both sets of experiments.

**Stimulating Electrode Implantation**

In the first set of experiments, a bipolar stimulating electrode (10-mm length; Plastics One, Roanoke, VA) was chronically implanted into the lateral hypothalamus. This site was chosen for use in the present study based on its reliability in producing self-stimulation behavior in rats (55). Under anesthesia produced by an Equithesin-like anesthetic cocktail (composed of 0.97 g of pentobarbital sodium and 4.25 g of chloral hydrate/100 ml distilled water; 2 ml/kg ip; University of Iowa Hospital Pharmacy, Iowa City, IA), rats were placed in a stereotaxic instrument, the scalp was incised, and the head was leveled between bregma and lambda. The electrode was implanted into the
lateral hypothalamus at 3.0 mm posterior to bregma, 1.7 mm lateral to midline, and 8.5 mm ventral to the skull surface. Three jeweler’s screws and dental acrylic were used to fix the electrode to the skull. Butorphanol (3 mg/kg, sc; Bristol-Myers Squibb, Princeton, NJ) was administered to the animals for postoperative analgesia, and they recovered for a minimum of 5 days before the collection of behavioral data.

Behavioral Training and Baseline Measurements

All electrical stimulation training and testing was carried out during the light period, between the hours of 0900 and 1600. Rats were trained in a Plexiglas operant conditioning chamber (Skinner Box) equipped with a lever. Each lever press delivered a negative-going, square pulse train lasting 200–500 ms, at 60 Hz, through the electrode. The training procedure consisted of first placing the rat into the operant chamber and allowing it to explore the environment for several hours. The electrical parameters (train duration, frequency, and current intensity) were set to predetermined values, and the experimenter gave the rat a few “free” electrical pulses. When the rat began to approach the lever, the parameters were systematically varied, and “free” pulses of current were administered until the rat began to respond for the stimulation by pressing the lever. Once the optimum parameters were determined for each rat, these were held constant throughout the entire study (with the exception of current intensity, which was systematically varied; these methods are described in the following paragraph). Rats that did not respond to electrical stimulation or that demonstrated marked motor effects to the stimulation that interfered with responding were not used in the study (i.e., this was a functional assessment of proper electrode placement).

After consistent response rates were established, current-response curves were determined for each rat using procedures similar to those described elsewhere (17, 40). Current was delivered in a descending series from 350 to 50 μA in discrete presentations of 25-μA decrements, and the animal was allowed to respond at each level of current for 1 min. An optimal current-response curve was generated for each rat using the following criteria: 1) the range of current intensities to which the rat responded was between 50 and 350 μA; 2) the response rate was minimal for low levels of current (e.g., ~50–100 μA), and it increased monotonically, eventually reaching a stable plateau during 10 consecutive presentations of 25-μA-increment current intensities, so that a sigmoid relationship between current intensity and behavioral responses was established; and 3) the maximum current intensity for which the rat responded did not produce untoward motor effects. Baseline current-response functions were generated over a 3- to 5-day period, and the results from different days were averaged for each rat.

Immediate Post-HU Behavioral Measurements

Within 2 h following removal from the HU or CC cage apparatus, anhedonia was assessed by generating current-response curves in HU and CC groups in the same manner as the baseline measurements. That is, current was delivered in a descending series from 350 to 50 μA in discrete presentations of 25-μA decrements, and the animal was allowed to respond at each level of current for 1 min.

Recovery of Behavioral Measurements following HU

Following the immediate post-HU and -CC electrical stimulation tests, both HU and CC rats were returned to a normal cage environment in the normal posture for a period of 7 days. All rats were subsequently tested for responses to electrical brain stimulation following this period. Current-response curves were generated in the same manner as the baseline and immediate post-HU and -CC measurements.

Sucrose Preference Tests

Sucrose preference tests were performed on animals in the second set of experiments. Rats were given access to sucrose solution (2%) intermittently for 1 wk preceding the experimental procedures to allow for adaptation to its taste. The methods for measuring sucrose preference used to operationally define anhedonia have been described previously (20, 53, 72). Briefly, 2% sucrose intake and water intake were measured following removal of food or water for 20 h before preference testing. Animals were allowed to consume fluids for a period of 1 h. Tests at baseline and on day 11 of the protocol were performed to measure sucrose preference before and during the HU or CC procedure. Anhedonia was defined as a reduction in sucrose intake and sucrose preference relative to the baseline and to that of the control group.

Catheter Implantation

In the second set of experiments, surgical implantation of femoral venous and arterial catheters was performed on day 12 of the HU or CC condition. Surgical procedures were carried out under halothane anesthesia, using aseptic surgical techniques. A polyethylene (PE 10 fused to PE 50) catheter was inserted into the aorta via the left femoral artery and vein for the measurement of arterial pressure and administration of autonomic blocking agents, respectively. The catheters were tunneled subcutaneously and exteriorized at the dorsal cervical region. Catheters were filled with heparinized saline (200 units/ml) and capped with an airtight plug when not in use. Animals were given butorphanol (3 mg/kg sc) for postoperative analgesia. Following immediate recovery from anesthesia, animals were returned to the HU or CC condition for an additional 48 h of recovery before collection of cardiovascular data.

Arterial Pressure Recordings

On the 14th day of the protocol in experiment 2, animals were removed from the HU apparatus and placed in the normal posture for cardiovascular experiments. Animals were given at least 1 h to acclimate to the new environment. Blood pressure recordings were carried out during the light period, between the hours of 0900 and 1600. Direct mean arterial pressure was recorded in unrestrained, unanesthetized rats. The catheter was connected to a pressure transducer (Maxxim Medical, Athens, TX) coupled to a multichannel recorder through a custom-designed amplifier (Department of Psychology Instrumentation Shop, University of Iowa, Iowa City, IA). The analog input was converted into a digital signal using a PowerLab data acquisition system (ADInstruments, Mountain View, CA). Mean arterial pressure was derived electronically online using the cyclic mean. The sampling rate was 1,000 samples/s. Heart rate was determined by measuring the number of heartbeats triggered from the arterial pressure pulse. Hemodynamic parameters were monitored for 30–60 min to ensure stabilization of mean arterial pressure and heart rate. After stabilization, baseline arterial pressure was recorded over a period of 1 h.

HRV Analysis

In experiment 2, HRV and spectral analysis of blood pressure was performed using the PowerLab chart extension module. A minimum of 40 min of stable arterial pressure, free from artifacts, was taken from the 1-h baseline arterial pressure recordings to evaluate the variations in heart period and perform spectral analysis of blood pressure. The systolic pulse recording was statistically analyzed by taking the standard deviation of all normal-to-normal (N-N) intervals from the systolic pulse waveform. The standard deviation of N-N intervals was calculated from baseline data in each individual rat [SDNN index, as described by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (68)]. The standard deviation of change in N-N interval, calculated on each beat, was also calculated (SD of ΔNN).
Spectral Analysis of Blood Pressure

Spectral analysis of arterial blood pressure was performed on the same baseline data used for calculation of HRV. The derivative of blood pressure was passed through a low-pass filter of 45 Hz. Data were resampled at the mean spectrum R-R interval, and this value was then subtracted from each resampled value to detrend the direct-current frequency component. The spectrum was calculated using fast Fourier transformation analysis on successive segments of 1,024 current frequency component. The spectrum was calculated using fast then subtracted from each resampled value to detrend the direct-

Cardiac Autonomic Blockade

With procedures similar to those previously employed (20), pharmacological autonomic blockade was used to assess the relative cardiac sympathovagal balance in CC (n = 11) and HU (n = 11) rats. In the second set of experiments, experiments could not be performed on one CC and one HU animal from each group due to occluded arterial catheters. Heart rate responses were measured under the following conditions: 1) during β-adrenergic receptor blockade with propranolol hydrochloride (2 mg/kg iv) followed by muscarinic cholinergic receptor blockade with atropine methylbromide (i.e., methylatropine 1 mg/kg iv) in one-half the rats in each group of CC (n = 5) and HU (n = 5) rats, 2) with the drug order reversed in the remaining one-half of rats in each group (i.e., methylatropine followed by propranolol; CC: n = 6; HU: n = 6), and 3) during complete autonomic blockade with propranolol + methylatropine in all rats. Since the response to complete autonomic blockade was not different relative to the order in which the drug was administered in each respective group, the responses were averaged. The drug doses were chosen for their ability to effectively block the respective autonomic inputs to the heart according to previous tests of efficacy. Data were calculated as the absolute effect on heart rate as well as the change in heart rate from baseline.

Plasma Corticosterone

Plasma levels of corticosterone were determined using a commercially available radioimmunoassay kit (MP Biomedicals, Irvine, CA). Plasma was diluted in assay buffer as necessary (1:200) to give results reliably within the linear portion of the standard curve. The inter- and intra-assay coefficients of variation for corticosterone are <5%, and cross reactivity with other steroids is <1%. The minimum detectable dose for this assay is 7.7 ng/ml.

Statistical Analysis

In experiment 1, current-response functions were calculated for each individual rat at different time points (baseline, immediate post-HU or -CC condition, and 7 days post-HU or -CC recovery). Data points from individual rats were plotted using Sigma Plot (Jandel Scientific, Chicago, IL), and the following three-parameter sigmoidal function was fit to the data:

\[
y = a/[1 + \exp(- (x - x_0)/b)]
\]

Mean current-response functions were calculated by averaging the response rate for each rat at each level of current intensity and by plotting the mean data using Sigma Plot. A three-parameter sigmoidal function was similarly fit to these data shown above where y = the number of lever presses per minute, x = the standardized current level; \(x_0\) = the EC_{50}; a = the maximum parameter and b = the minimum parameter.

From each individual fit curve, three parameters were calculated: 1) threshold or current intensity which supported 50% of the maximum response rate (\(x_0\) defined as the effective current “50”; \(EC_{50}\), 2) maximum rate of responding and corresponding current intensity (a), and 3) minimum rate of responding and corresponding current intensity (b). Mean \(EC_{50}\) and maximum response values were statistically compared in HU and CC groups using mixed-design ANOVA and Student’s t-tests, and a Bonferroni correction for relevant multiple comparisons. Cardiac autonomic blockade data were analyzed using repeated-measures ANOVA.

In both sets of experiments, changes in body weight were compared using two-way ANOVA with repeated measures design, as were absolute water and sucrose intake in experiment 2. Absolute change in body weight, soleus and plantaris muscle wet weights and muscle weight to body weight ratios were compared using independent t-tests. For all statistical analyses in both sets of experiments, a probability value <0.05 was considered to be statistically significant.

RESULTS

Experiment 1

HU. Body weights before, immediately after, and following 7 days of recovery from the HU or CC condition are shown in Table 1. Body weight was significantly lower in HU animals 14 days following HU and after 7 days of recovery from HU (P < 0.05). Both soleus and plantaris wet muscle weights and soleus and plantaris muscle-to-body weight ratios measured following 7 days of recovery from HU were not significantly different between groups.

Behavioral responses to electrical stimulation following HU. A sigmoidal current-response relationship between current intensity and response rate was observed for rewarding electrical brain stimulation. As current intensity increased, response rates increased and reached an asymptote. HU resulted in reduced responding for electrical stimulation across a range of current intensities, relative to baseline responding and to the responses of CC rats (Figs. 1 and 2). A parallel rightward shift was observed in the current-response function in the HU group, compared with baseline (Fig. 2B) and CC group responses (Fig. 2A).

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>Soleus Wt 7-day Recovery, mg</th>
<th>Plantaris Wt 7-day Recovery, mg</th>
<th>Soleus/BW × 10³, mg/g</th>
<th>Plantaris/BW × 10³, mg/g</th>
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</thead>
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<tr>
<td></td>
<td>n</td>
<td>Baseline</td>
<td>Post-HU/CC</td>
<td>7-day Recovery</td>
<td>Baseline</td>
</tr>
<tr>
<td>Casted control</td>
<td>6</td>
<td>333 ± 7.4</td>
<td>358 ± 14.4</td>
<td>378 ± 13.6</td>
<td>131 ± 0.01</td>
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<tr>
<td>Hindlimb unloaded</td>
<td>6</td>
<td>322 ± 2.2</td>
<td>316 ± 6.9*</td>
<td>329 ± 8.4*</td>
<td>110 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. HU, hindlimb unloading; BW, body weight. *P < 0.05 vs. casted control group.
Mean curve parameters, including the maximum, EC₅₀, and minimum for both groups at baseline, immediately following, and after recovery from the HU or CC condition, are shown in Fig. 1. There were no significant differences with respect to minimal parameters at any time point. The baseline EC₅₀ values were not different between HU and CC rats. However, immediately following 14 days of HU, the HU rats displayed a significantly higher mean EC₅₀ than its respective baseline value (P < 0.05) and the CC group (P < 0.05). In the CC group, the mean EC₅₀ value did not differ significantly from its respective baseline value after 14 days of the CC condition.

A mixed-design ANOVA yielded a significant main effect of group (P < 0.05) but no significant interaction for the maximum response rate in HU and CC rats.

Behavioral responses to electrical stimulation following recovery from HU. Electrical responses to ICSS were able to be maintained through a 7-day recovery period in all CC and HU rats. Additional analysis was performed on these animals to determine the extent of ICSS recovery after the HU or CC condition. Data indicate that HU produces a rightward shift in the current-response relationship to ICSS and that this effect fully recovers following 7 days after HU is terminated.

**Experiment 2**

**HU.** The effects of HU on body weight and hindlimb muscle weight are shown in Table 2. Similar to results in experiment.
1, body weight was significantly higher in CC rats compared with HU rats immediately following 14 days of the protocol. Both soleus and plantaris wet muscle weights and soleus and plantaris muscle-to-body weight ratios measured after 14 days of the HU or CC condition were significantly lower in HU rats ($P < 0.05$).

**Sucrose preference.** Although two-way ANOVA for repeated measures revealed no significant differences in absolute water intake (CC, day 1: 3.2 ± 0.5 ml; CC, day 11: 2.9 ± 0.3 ml vs. HU, day 1: 3.0 ± 0.2 ml; HU, day 11: 4.4 ± 0.5 ml), there was a significant group, day, and group × day interaction in absolute sucrose intake. Post hoc analysis revealed no significant difference in absolute baseline sucrose consumption between groups (CC, day 1: 8.4 ± 0.8 ml vs. HU, day 1: 7.8 ± 1.2) and a significant reduction in absolute sucrose intake on day 11 of the protocol (CC, day 11: 13.3 ± 1.4 ml vs. HU, day 11: 6.9 ± 0.9 ml). Data illustrating normalized sucrose preference at baseline and on day 11 of the protocol in HU and CC rats is shown in Fig. 3. Although there was no significant difference at baseline between CC and HU rats (Fig. 3A), HU rats exhibited a significant reduction in the relative amount of sucrose as a percentage of total fluid consumed on day 11 of the HU procedure ($P < 0.05$; Fig. 3B). Although the absolute intake of sucrose increased in control animals over the course of the protocol, there was no difference in CC rats at baseline and on day 11 of the CC procedure with regard to percent preference for sucrose.

**Plasma corticosterone.** Data comparing plasma corticosterone levels between CC and HU rats are shown in Table 3. There was no significant difference in plasma corticosterone levels between CC and HU rats measured at death on the 14th day of the HU or CC procedure (Table 3).

**Resting baseline hemodynamic parameters.** Baseline cardiovascular parameters following 14 days of the HU or CC condition are shown in Table 3. HU elicited no significant change in resting mean arterial pressure, although there was a significant elevation in resting heart rate compared with CC rats ($P < 0.05$). Two indices of HRV were calculated in the HU and CC rats. Both the SDNN Index and the SD of ΔNN was significantly reduced following HU, relative to the CC group ($P < 0.05$; Table 3).

**Spectral analysis of arterial blood pressure.** Results from a spectral analysis of arterial blood pressure in both groups of rats following 14 days of the HU or CC condition are shown in Table 4. The spectral analysis revealed that HU rats had a significant reduction in total power and absolute LF power and normalized LF power ($P < 0.05$). Although absolute HF power was reduced in HU rats, compared with CC rats ($P < 0.05$), there was no significant difference in normalized HU power.

**Cardiac autonomic blockade.** Data indicating the effects of cardiac autonomic blockade following HU are shown in Table 5 and Fig. 4, A and B. Baseline heart rate was significantly higher in HU vs. CC rats ($P < 0.05$; Tables 2 and 5; Fig. 4A). Administration of propranolol alone in 1/2 of the HU (n = 6) and CC (n = 6) rats to block cardiac β-adrenergic receptors (cardiac sympathetic tone) resulted in a greater decrease in heart rate in HU rats compared with CC rats ($P < 0.05$; Table 5, Fig. 4B). The steady-state heart rate recorded following administration of propranolol was not different between groups in HU vs. CC rats ($P < 0.05$; Tables 2 and 5; Fig. 4A).

**Table 2. Body weight and muscle weights in casted control and hindlimb unloaded groups in experiment 2**

<table>
<thead>
<tr>
<th></th>
<th>Casted control</th>
<th>Hindlimb unloaded</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Baseline</td>
<td>Post-HU/CC</td>
<td>Soleus Wt Post-HU, mg</td>
<td>Plantaris Wt Post-HU, mg</td>
<td>Soleus/BW × 10³, mg/g</td>
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<tr>
<td>Casted control</td>
<td>12</td>
<td>297±2.1</td>
<td>306±3.1</td>
<td>130±0.00</td>
<td>343±0.01</td>
<td>0.43±0.01</td>
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<tr>
<td>Hindlimb unloaded</td>
<td>12</td>
<td>292±2.1</td>
<td>271±3.3*</td>
<td>70.9±0.01*</td>
<td>271±0.01*</td>
<td>0.26±0.02*</td>
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Values are means ± SE; n, no. of rats. *$P < 0.05$ vs. casted control group.
(Table 5, Fig. 4A). The response to administration of methylatropine alone to block cardiac muscarinic receptors (cardiac parasympathetic tone) in the remainder of HU (n = 5) and CC (n = 5) rats resulted in a significantly greater increase in heart rate from baseline in CC rats and no significant change in heart rate from baseline in HU rats (P < 0.05; Table 5, Fig. 4B). The steady-state heart rate recorded following cardiac parasympathetic blockade was not different between the groups.

In all rats in both groups, following administration of the first autonomic blocker the second drug was administered to achieve complete cardiac autonomic blockade, thus enabling the measurement of intrinsic heart rate. In both groups of rats, absolute intrinsic heart rate was not different between groups but was significantly lower than baseline heart rate when data from the subgroups of rats were combined (P < 0.05; Fig. 4A). However, the decrease in heart rate from baseline was significantly greater in HU rats compared with CC rats (P < 0.05; Table 5, Fig. 4B).

In CC rats, the change in heart rate in response to propranolol in the presence of atropine was significantly greater than the response to propranolol alone (P < 0.05; Table 5). Although the response to atropine in the presence of propranolol was smaller in CC animals this effect did not reach statistical significance. The heart rate response was not altered by the order of drug administration in either HU group.

**DISCUSSION**

In the present study, we examined the hypothesis that the HU rat model of cardiovascular deconditioning would be accompanied by both anhedonia and cardiovascular autonomic imbalance. We performed two separate experiments to more fully test this hypothesis. Data indicate that HU results in anhedonia when measured either during or immediately after termination of HU. This was evidenced by both reduced behavioral responding for ICSS and reduced sucrose preference. Thus, with two different experimental methods, converging evidence indicates that HU produces anhedonia. In addition, the experimentally-induced cardiovascular deconditioning is also associated with altered cardiac sympathovagal balance, reduced HRV, and altered spectral analysis of blood pressure.

Anhedonia, defined as the reduced responsiveness to pleasurable stimuli, is estimated to be present in ~95% of all depressed patients (34), and this symptom is a core feature of Major Depressive Disorder according to *Diagnostic and Statistical Manual of Mental Disorder, 4th Edition, Text Revision* criteria (1). Data from the present study indicate that cardiovascular deconditioning produced through hindlimb unloading induces anhedonia. Two separate specific hedonic measures were obtained which led to this conclusion. In experiment 1 a greater EC50 was present in HU rats immediately following 14 days of HU compared with their own baseline and the CC group. This value indicates a rightward shift in the current-response function, such that responding is reduced at the same level of current that previously supported the responses and a greater current intensity is required to produce the same level of previously recorded responding (Fig. 1). Parallel shifts in current- or frequency-response functions in electrical brain stimulation paradigms have been suggested as evidence for a change in the reinforcing efficacy of the electrical stimulation (40). Furthermore, the EC50 in HU rats returned to baseline levels following a 7-day recovery in the normal posture. This recovery value was not significantly different from the HU animals’ original baseline value or that of CC animals (Fig. 2).

Although the maximum response rate to electrical stimulation was not significantly altered in response to HU, animals that recovered from HU did have a tendency toward an increased maximal response. This may indicate that HU rats had improved motor performance following recovery. This response is not surprising considering that HU rats are required to increase forelimb use during the HU procedure and thus they may have improved motor capacity as a result. In addition, any attenuation in ICSS reward could not be attributable to reduced motor performance in this group; in fact, just the opposite could be argued. These conclusions regarding use of these curve parameters as a measure of ICSS reward properties are well supported by previous research employing identical methods in a number of models and task-dependent manipulations (17, 40, 47).

Further evidence for anhedonia in HU rats is supported by the observation that sucrose preference was reduced on day 11 of the 14 day HU protocol. Sucrose preference was not signif-

### Table 3. Baseline cardiovascular parameters and plasma corticosterone concentration in casted control and hindlimb unloaded groups in experiment 2

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>MAP, (mmHg)</th>
<th>HR, beats/min</th>
<th>SDNN, ms</th>
<th>SD ΔNN, ms</th>
<th>Plasma Corticosterone, µg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casted control</td>
<td>11</td>
<td>121±3.9</td>
<td>383±9.6</td>
<td>10.4±1.4</td>
<td>3.43±0.4</td>
<td>11.1±1.4</td>
</tr>
<tr>
<td>Hindlimb unloaded</td>
<td>11</td>
<td>128±3.0</td>
<td>462±9.1*</td>
<td>6.96±0.7*</td>
<td>2.48±0.2*</td>
<td>11.0±2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; MAP, mean arterial pressure; HR, heart rate; SDNN, standard deviation of normal-to-normal (NN) intervals; SD ΔNN, standard deviation of change in NN intervals. *P < 0.05 vs. casted control group.

### Table 4. Spectral analysis of blood pressure at baseline following 14 days of casted control and hindlimb unloading in experiment 2

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Total Power, ms²</th>
<th>LF, ms²</th>
<th>HF, ms²</th>
<th>LF, nu</th>
<th>HF, nu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casted control</td>
<td>11</td>
<td>28.9±4.38</td>
<td>3.48±0.87</td>
<td>3.55±0.78</td>
<td>42.02±5.57</td>
<td>45.97±4.79</td>
</tr>
<tr>
<td>Hindlimb unloaded</td>
<td>11</td>
<td>10.8±1.86*</td>
<td>0.58±0.09*</td>
<td>1.59±0.25*</td>
<td>19.34±2.54*</td>
<td>48.76±3.38</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. LF, low frequency; HF, high frequency; nu, normalized units. *P < 0.05 vs. casted control group.
significantly different between CC and HU groups at baseline. The use of sucrose preference as a specific measure of anhedonia has been used extensively in a wide range of depressive animal models and is indicative of the reduced responsiveness to pleasurable stimuli (anhedonia) often observed in human depression (1, 34). The reduced preference for sucrose during the later stages of HU was specific to the procedure and not due to attenuated ingestive behaviors since HU rats did not consume less water than CC animals when preference was measured on day 11 of the protocol. In addition, a second test of sucrose preference in CC animals revealed no significant reductions; thus the attenuation in preference in HU rats is not attributable to a reduced preference as a function of repeated exposure to sucrose.

Psychological depression is characterized by depressed mood, anhedonia, fatigue and physical inactivity (1). Although adverse psychological changes following bed rest or microgravity in humans have been previously documented (25, 27, 33, 66), whether cardiovascular deconditioning leads to psychological depression is not commonly reported. In the present study, we used the HU animal model to more systematically evaluate the hypothesis that cardiovascular deconditioning is associated with depression and cardiovascular autonomic dysfunction.

Data from the present studies indicate that HU results in a profound change in sympathovagal balance. This conclusion is supported from multiple observations. Results from experiment 2 indicate that although there is no change in resting mean arterial pressure, HU rats exhibit a resting tachycardia and reduced HRV (Table 3). Data from cardiac autonomic blockade indicate that these effects are due to both an increased cardiac sympathetic tone and near absence of cardiac vagal tone in the HU rats (Table 5, Fig. 4). This is evidenced by the observation that cardiac sympathetic blockade by administration of propranolol resulted in a greater decrease in heart rate in HU vs. CC rats. In addition, pharmacological blockade of parasympathetic tone to the heart by administration of methy- latropine did not significantly increase heart rate above baseline in HU rats, while heart rate increased by 59 ± 14.9 beats/min in CC rats. We did observe that the heart rate response to propranolol was significantly greater following atropine administration in the CC group (Table 5). This is likely due to accentuated antagonism from presynaptic cholinergic inhibition of norepinephrine release from cardiac sympathetic terminals (35). It is interesting to note that this effect was only observed in CC animals, which emphasizes the lack of vagal tone to the heart in HU animals.

These findings taken together indicate that sympathovagal balance is altered in response to HU such that there is both a significant increase in sympathetic tone and profound reduction in parasympathetic tone to the heart. This finding is in agree-
ment with Mueller et al. (48) in which HU rats were reported to have a blunted increase in heart rate in response to parasympathetic blockade by atropine, and enhanced reductions in heart rate and mean arterial pressure in response to ganglionic blockade via hexamethonium. In addition, reduced parasympathetic and increased sympathetic cardiac tone has been reported in astronauts following exposure to microgravity (39).

Additional findings from the present study indicate that autonomic tone to the vasculature is altered by HU. Power spectral analysis of arterial blood pressure revealed that HU resulted in a significantly reduced total power, absolute HF power, and both absolute and normalized LF power ($P < 0.05$; Table 4). Only normalized HF power was not significantly different between CC and HU rats. There are several possible interpretations of this finding. Because blood pressure and sympathetic nervous system activity have been found to be tightly coupled at 0.4 Hz (within the LF band designated in the present study; Refs. 5, 8), in general, changes in power from 0.2 to 0.8 Hz have been used to indicate changes in vasomotor sympathetic drive, although not without exception (57). Thus, one interpretation of the spectral analysis data from the present study is that HU resulted in reduced sympathetic vascular tone. This interpretation, however, would be in disagreement with the current finding that HU results in augmented cardiac sympathetic tone, as well as previous data indicating that HU rats have a greater resting sympathetic tone to the vasculature (48). In addition, the use of changes in power from 0.2 to 0.8 Hz as an index of chronic sympathetic tone to the vasculature has been called into question by data indicating that a higher level of directly measured splanchnic sympathetic nerve activity was not associated with higher blood pressure power at 0.2–0.8 Hz (65). The most plausible interpretation for the current data is that reduced total power and greatly reduced LF power is reflective of reduced baroreflex control of sympathetic nervous system activity to the vasculature. This explanation is consistent with previous studies indicating that baroreflex control of sympathetic nerve activity is attenuated following HU in rats (12, 41, 44, 45) and that sympathetic responses to orthostatic stress are attenuated in humans (31, 62). Furthermore, this finding is consistent with the observation that LF power (0.27–0.74 Hz, similar to the LF band used in the present study) is reduced, while HF power is preserved following sinoaortic baroreceptor denervation in rats (8, 28, 29). The authors noted that the presence of the well-described LF peak at $\sim0.4$ Hz was abolished following sinoaortic baroreceptor denervation. It is interesting to note that in the present study this peak was clearly observed in 9 of 11 CC rats, while this peak was detectable only in 4 of 11 HU rats and at a much reduced amplitude of power than in CC rats. This effect has been substantiated in humans in that the sensitivity of the gain of the baroreflex is positively correlated with LF power (10, 64). Thus the reduction in LF power in HU rats observed in the present study may be related to the attenuation in baroreflex control of sympathetic nervous system activity that has been previously well described in the HU rat model (12, 41, 44, 45).

Although absolute power in the HF band was reduced following HU ($P < 0.05$; Table 5), there was no difference in normalized HF power in HU vs. CC rats. Although HF blood pressure power may be indicative of cardiac vagal drive, this index is controversial and is most consistently associated with mechanical effects related to respiration (57). Thus this finding may indicate that HU rats have altered respiration, although we did not measure the respiratory rate in HU rats. In addition, normalized HF power was not altered, which is an index normalized to total power minus very LF power (0.0–0.195 Hz). We did not believe that the very LF power could be legitimately evaluated in this study due to the relatively short ($<1$ h) recording period. However, because HU resulted in a greatly reduced very LF power (data not shown), this effect could have served to result in no change in normalized HF power.

Data from the present study indicate that HU results in elevated cardiac sympathetic nervous system activity. It is unknown if there is a generalized increase in sympathetic outflow, which may be indicative of increased stress associated with this model. In the present study, we found no significant differences in plasma corticosterone levels following 14-days of HU or CC condition. This finding is in agreement with previous data indicating that 14 days of HU does not induce hypertrophy of the adrenal glands (12). These values are similar to those measured using similar collection techniques in control animals during the evening hours when corticosterone levels are at their peak (23, 36, 38). These data indicate that any elevation in sympathetic tone is most likely due to specific physiological adjustments associated with cardiovascular deconditioning (i.e., compensation for reduced blood and plasma volume or autonomic dysregulation) rather than a generalized, nonspecific stress response to the HU paradigm.

Psychological depression has a bidirectional association with cardiovascular disease indicating that the presence of one of these conditions increases an individual’s likelihood of developing the other disorder (18). Previously, our laboratory demonstrated the presence of both anhedonia and autonomic imbalance in the chronic mild stress rodent model of depression (20), as well as altered sympathoexcitatory responses to behavioral and pharmacological stimuli in the olfactory bulbectomy depression model (42). Although the association between depression-related signs and autonomic imbalance has been repeatedly demonstrated, the precise physiological mechanisms that underlie this relationship have yet to be understood. It is possible that the link between depression and cardiovascular dysfunction may be mediated in part by autonomic imbalance and dysregulation of autonomic reflexes that serve to regulate cardiovascular function. Reduced HRV, increased sympathetic tone, and reduced baroreflex sensitivity have been observed in depressed patients (2, 58, 70, 71) with an increase in HRV found with successful treatment for depression (2).

Previous studies in humans following bed rest and microgravity have indicated that cardiovascular deconditioning is associated with depression (25, 33, 66) and disordered autonomic tone (3, 39), although no studies to our knowledge have systematically explored the interrelationship between these factors. The present study describes the presence of both depressive-like signs and sympathovagal imbalance in an animal model of cardiovascular deconditioning. It is possible that HU leads to sympathovagal imbalance, similar to the imbalance that is observed in depressed patients and in experimental rat models of depression (18, 70). This sympathovagal imbalance may in turn affect the behavioral and cardiovascular changes in these rats. Although the mechanisms responsible for this effect are unknown, underlying changes within the central
nervous system are likely. Likely candidates include changes in serotonin and/or catecholamines (i.e., norepinephrine and/or dopamine) neurotransmitter systems. Anhedonia was reversed and cardiovascular dysfunction was partially reversed following treatment with fluoxetine in the chronic mild stress rat model of depression (16). In addition, short-term fluoxetine treatment reversed attenuated baroreflex control of sympathetic nervous system activity following 14-days of HU in rats (44).

HU produces a significant reduction in plasma volume (67), similar to what is observed in humans following prolonged bed rest or exposure to microgravity (9). Elevations in volume regulating hormones, which are thought to help compensate for this contracted circulating volume, have been measured both during and after HU. Although changes in plasma renin activity have not been found to be altered either during or after HU (50, 67), elevations in plasma aldosterone have been observed during and after HU. Although changes in plasma renin activity have not been found to be altered either during or after HU (50, 67), elevations in plasma aldosterone have been observed within 24 h (67), and after 7 days (52) of HU. Interestingly an increase in aldosterone, in the absence of any changes in plasma renin activity, was measured in two separate studies of clinically depressed patients (11, 51). In addition, aldosterone has been implicated in both the anhedonic state in sodium-deprived rats (47) as well as attenuated baroreflex control of sympathetic nerve activity in heart failure rats (13). Thus, although aldosterone may be a critical link between changes in affect and sympathovagal balance following cardiovascular deconditioning, future studies are needed to address this hypothesis.

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GRANTS

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