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Baroreflex control of muscle sympathetic nerve activity after 120 days of 6° head-down bed rest

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Kamiya, Atsunori, Satoshi Iwase, Hiroki Kitazawa, Tadaaki Mano, Olga L. Vinogradova, and Irina B. Kharchenko. Baroreflex control of muscle sympathetic nerve activity after 120 days of 6° head-down bed rest. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 278: R445–R452, 2000.—To examine how long-lasting microgravity simulated by 6° head-down bed rest (HDBR) induces changes in the baroreflex control of muscle sympathetic nerve activity (MSNA) at rest and changes in responses of MSNA to orthostasis, six healthy male volunteers (range 26–42 yr) participated in Valsalva maneuver and head-up tilt (HUT) tests before and after 120 days of HDBR. MSNA was measured directly using a microneurographic technique. After long-term HDBR, resting supine MSNA and heart rate were augmented. The baroreflex slopes for MSNA during Valsalva maneuver (in supine position) and during 60° HUT test, determined by least-squares linear regression analysis, were significantly steeper after than before HDBR, whereas the baroreflex slopes for R-R interval were significantly flatter after HDBR. The increase in MSNA from supine to 60° HUT was not different between before and after HDBR, but mean blood pressure decreased in 60° HUT after HDBR. In conclusion, the baroreflex control of MSNA was augmented, whereas the same reflex control of R-R interval was attenuated after 120 days of HDBR.

microneurography; orthostatic hypotension

ORTHOSTATIC HYPOTENSION IS common after exposure to real and simulated microgravity (3–6, 10–12, 14–20, 26, 30, 34, 38). This condition may be produced after ground-based simulation of microgravity, such as 6° head-down bed rest (HDBR) in humans (10–12, 14, 18, 20, 22, 24, 30, 38). Microgravity and HDBR rapidly induce a fluid shift to the central part of the body, which may elicit cardiovascular and neurohumonal effects that promote natriuresis and diuresis, resulting in a sustained decrease in circulatory blood volume (4, 5, 34). The loss of plasma volume after exposure to microgravity has been thought to contribute to orthostatic hypotension, but restoration of plasma volume does not completely rectify the orthostatic intolerance (4). The mechanisms of orthostatic hypotension remain unclear, but several studies demonstrated and speculated changes in neural regulation, attenuated baroreflex control of heart rate (10, 15–17, 19), and impairment of cerebral vascular flow autoregulation (38) in particular, as contributing factors. Recent studies have reported that changes in the regulation of peripheral vasculatures might relate to orthostatic hypotension after exposure to microgravity (6, 18, 35). Fritsch-Yelle et al. (18) documented that attenuated increases in plasma norepinephrine concentrations might contribute to orthostatic hypotension after microgravity. A recent rodent study showed that baroreflex modulation of sympathetic nerve activity to both the skeletal muscle and the kidney declined after 14 days of hindlimb unloading (26). However, the baroreflex control of vasomotor sympathetic nerve activity, which regulates peripheral vascular resistances, has not been examined in humans.

The arterial baroreflexes are important mechanisms for the overall regulation of blood pressure during orthostatic stress and exercise (13). Under orthostasis, the baroreflex mediates tachycardia and peripheral vasoconstriction to lessen gravity-induced fluid shift and to preserve arterial blood pressure. Peripheral vascular resistance is under baroreflex control via vasomotor sympathetic nerve activity, which plays a most important role in the maintenance of arterial blood pressure during orthostasis (13, 21, 27). Therefore, dysfunction of baroreflex control of vasomotor sympathetic nerve activity can contribute both to orthostatic intolerance and decreased upright exercise performance after exposure to real and simulated microgravity (13, 21, 27).

We hypothesized that the impaired baroreflex control of vasomotor sympathetic outflow to peripheral vessels may contribute to orthostatic hypotension after exposure to microgravity in humans. To examine long-lasting simulated microgravity-induced changes in the baroreflex control of vasomotor sympathetic nerve activity and in the responses of vasomotor sympathetic nerve activity to orthostasis, Valsalva maneuver and head-up tilt (HUT) tests were performed before and after 120 days of 6° HDBR. Vasomotor sympathetic discharges were recorded directly as muscle sympathetic nerve activity (MSNA) using a microneurographic technique.

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METHODS

Subjects. Six healthy Russian male volunteers with a mean age of 31 ± 3 (SE) yr (range 26–42 yr), mean height of 180 ± 2 cm (range 175–190 cm), and mean weight of 80 ± 8 kg (range 63–114 kg) were studied. All subjects were evaluated to have normal physical fitness by detailed medical history, physical examination, complete blood count, urinalysis, resting and treadmill electrocardiograms, a panel of blood chemistry analyses, and psychological tests. No subject smoked, used recreational drugs, or had chronic medical problems. All subjects gave informed consent to participate in the study, which was approved by the Ethical Committee of Institute of Biomedical Problems, State Scientific Center, Moscow, Russia.

HDBR protocol. The experiment was carried out at the Institute of Biomedical Problems, State Scientific Center, Moscow, Russia, during the period between December, 1996 and June,1997. Each subject was exposed to 120 days of strict adherence to 6° HDBR. Valsalva maneuver and HUT tests were performed 30 days before HDBR (Pre-HDBR), on *days 60* and *120* of HDBR ($HDBR_{60}$ and $HDBR_{120}$, respectively), and after 30 days of recovery from HDBR (Rec-HDBR). During HDBR, the subjects were allowed to sit up on the bed for elimination, only for 15 min/day. Dietary intake was 2,300 to 2,500 kcal/day, and water intake was 1.0 to 1.5 l/day. Smoking and drinking caffeinated beverages were strictly prohibited throughout the course of the experiment.

Measurements. The MSNA was recorded by microneurography from the tibial nerve of one leg, with monitoring of the electrocardiograms from chest lead II and respiration using a thermistor. Brachial blood pressure was measured every minute as an index of systemic blood pressure. Beat-to-beat arterial blood pressure was monitored noninvasively using a Finapres 2300 (Ohmeda, Englewood, CO). The finger cuff of the Finapres was attached to a digit of the right hand at the height of the heart level. All variables except intermittent brachial blood pressure measurements were stored on a DAT recorder (PC216Ax, Sony Magnescale, Japan) for further analysis.

Microneurography. A Tungsten microelectrode with a shaft diameter of 120 μm and an electrode impedance of 2 to 5 MΩ (model 26–05–1, Federick Haer and Co., Brunswick, ME) was inserted percutaneously into the muscle nerve fascicles of the tibial nerve at the popliteal fossa without anesthesia. Nerve signals were fed into a high input-impedance preamplifier (laboratory-handmade, input impedance: 100 MΩ; gain: 40,000) with two active band-pass filters set between 500 and 5,000 Hz and were monitored with a loudspeaker. MSNA was identified according to the following discharge characteristics: pulse synchronous spontaneous efferent discharges; afferent activity being evoked by tapping of the soleus muscle but not in response to a gentle skin touch; and enhancement during phase II of Valsalva maneuver.

At supine rest. The subjects rested in supine position on a tilt table for >30 min before the experiment began. Resting supine recordings of MSNA as well as of electrocardiograms, brachial blood pressure, finger blood pressure, and respiration were performed for 20 min (Table 1). The recording began at least 10 min after obtaining successful recording of MSNA.

Valsalva maneuver. Valsalva maneuver at 40 mmHg expired pressure was performed in a supine position. Each straining period lasted 20 s. All measurements were made during period II of the Valsalva maneuver (see Figs. 1 and 2).

HUT test. After at least 7 min of supine (0°) rest, the tilt table was inclined to 30° and 60° in a passive and graded manner. Each position was fixed for 5 min. The HUT test was terminated for return to the supine position after any of the following: completion of 60° of HUT, onset of presyncopal symptoms such as nausea, sweating, grayout, or dizziness with a drop in systolic blood pressure >15 mmHg and/or sudden bradycardia >15 beats/min, and progressive reduction in systolic blood pressure to <80 mmHg (13, 29).

Table 1. *MSNA, heart rate, and mean blood pressure*

	Pre-HDBR	$HDBR_{60}$	$HDBR_{120}$	Rec-HDBR
MSNA burst rate, bursts/min	19.0 ± 2.3	28.2 ± 2.7*	29.4 ± 4.2*	23.0 ± 3.3
Heart rate, beats/min	65.1 ± 2.3	73.0 ± 2.2*	72.1 ± 2.9*	67.0 ± 2.9
Mean blood pressure, mmHg	86.3 ± 2.2	89.1 ± 2.3	89.4 ± 2.2	89.8 ± 1.1

Muscle sympathetic nerve activity (MSNA), heart rate, and mean blood pressure during 20 min of resting control period in supine position before (Pre-HDBR), after 60 and 120 days of head-down bed rest ($HDBR_{60}$ and $HDBR_{120}$, respectively), and during recovery (Rec-HDBR). For Pre-HDBR, $HDBR_{60}$, and $HDBR_{120}$, $n = 6$; for Rec-HDBR, $n = 3$. Values are means ± SE. *$P < 0.05$ vs. Pre-HDBR. We did not perform statistical analysis of data on Rec-HDBR due to small sample number.

Data analysis. MSNA was full-wave rectified and fed through a resistance-capacitance integrating circuit with a time constant of 0.1 s to obtain the mean voltage neurogram, which was displayed along with the electrocardiograms, blood pressure, and respiration on a pen recorder (RECTI-HORIZ, NEC San-Ei, Tokyo, Japan). MSNA was expressed as *1*) MSNA burst rate, i.e., the mean number of sympathetic bursts per minute, *2*) MSNA burst amplitude, and *3*) total MSNA, i.e., the sum of the MSNA burst amplitudes of all bursts for each analyzed period per minute. For calculation of MSNA burst, amplitude in the mean voltage neurogram, all burst amplitudes were measured using a paper sheet. As the MSNA burst amplitude was dependent on electrode position, which varied from day to day, the mean MSNA burst amplitude during 20 min of resting supine control was given the arbitrary value of 100, and all other MSNA burst amplitudes during Valsalva maneuver and each 5 min of supine (0°), 30°, and 60° in HUT tests were expressed in relation to this value. We observed an elevated baseline during Valsalva maneuver (see Figs. 1 and 2), but measured burst amplitude during the maneuver from the pre-Valsalva baseline level, not from the start of each individual burst. Therefore, the elevated baseline was not an artifact due to muscle tension during straining, because it remained unchanged with two active band-pass filters set between 1,500 and 5,000 Hz, which eliminate an electromyogram from microneurographic signal. Thus we expressed MSNA data during 20 min of resting supine control as only MSNA burst rate, those during Valsalva maneuver as MSNA burst amplitude, and those during each stage of HUT test as MSNA burst rate, MSNA burst amplitude, and total MSNA.

The beat-to-beat data for R-R interval and systolic and diastolic blood pressure were obtained by detecting R-wave peaks and by identifying peaks and troughs on the blood pressure wave, respectively. Mean blood pressure was calculated as diastolic blood pressure plus one-third of pulse pressure, a difference between systolic and diastolic blood pressure.

The periods for analyses were 20 min of resting control in supine position followed by Valsalva maneuver and each

5 min of supine (0°), 30°, and 60° of HUT positions during HUT tests of each subject every day.

Baroreflex slopes during Valsalva maneuver. The baroreflex slope for MSNA and heart rate during the early period of blood pressure fall in phase II of Valsalva maneuver was determined for each subject by least-squares linear regression analysis according to a previous study (25). Each R-R interval and each MSNA burst amplitude (arbitrary units) obtained during early phase II was related to the corresponding systolic and diastolic blood pressure, respectively. The period for analysis was confined to the period of mean blood pressure decrease from a peak to a trough in early phase II (see Fig. 1). These slopes were defined as the baroreflex function. In most studies, we observed only one or two MSNA bursts during the period of blood pressure rise in phase IV. Thus it might be difficult to obtain a satisfactory baroreflex slope for MSNA in phase IV, and we presented only baroreflex slopes in early phase II (blood pressure-fall period).

Baroreflex slopes in supine (0°) and 60° of HUT tests. The baroreflex slopes for MSNA in supine (0°) and 60° HUT positions were determined for each subject according to a previous study (31). Beat-by-beat values for MSNA burst amplitude (arbitrary units) were averaged over 2 mmHg diastolic blood pressure ranges, and a weighted linear regression between nerve activity and pressure was performed. These slopes were defined as the baroreflex function of MSNA.

The baroreflex slopes for heart rate in supine (0°) and 60° HUT positions were obtained by applying the sequence analysis introduced by Bertinieri et al. (2). More than three consecutive beats in which the systolic blood pressure and R-R interval changed in the same direction were searched for in the time-series data as baroreflex sequence. The linear regression line was processed to each baroreflex sequence by the least-squares method. The arterial baroreflex sensitivity was defined as the average value of all slopes within each given period.

Statistical analysis. Data are expressed as means ± SE. The Friedman's test followed by a post hoc test (Bonferroni-Dunn comparison procedure) was performed to examine the effect of HDBR on MSNA, heart rate, mean blood pressure, and barorefrex slope for MSNA and heart rate and the effect of HUT on MSNA, heart rate, and mean blood pressure on each trial. For the determinations of baroreflex slopes, the statistical criteria for the significant correlation was that the correlation coefficient (r) is >0.80 with $P < 0.05$ by Fisher's r to z (P value). The $P < 0.05$ level of differences was considered significant.

RESULTS

At supine rest. Resting supine MSNA burst rate was significantly greater on $HDBR_{60}$ and $HDBR_{120}$ (28.2 ± 2.7 and 29.4 ± 4.2 bursts/min, respectively) than on Pre-HDBR (19 ± 2.3 bursts/min). MSNA burst rate returned to the Pre-HDBR level on Rec-HDBR (Table 1).

Heart rate at supine rest elevated on $HDBR_{60}$ and $HDBR_{120}$ (73.0 ± 2.2 and 72.1 ± 2.9 beats/min, respectively) compared with that on Pre-HDBR (65.1 ± 2.3 beats/min). Mean blood pressure at supine rest did not change during 120 days of HDBR nor on Rec-HDBR (Table 1).

Valsalva maneuver. Figures 1 and 2 show baroreflex slopes for MSNA and R-R intervals during early phase

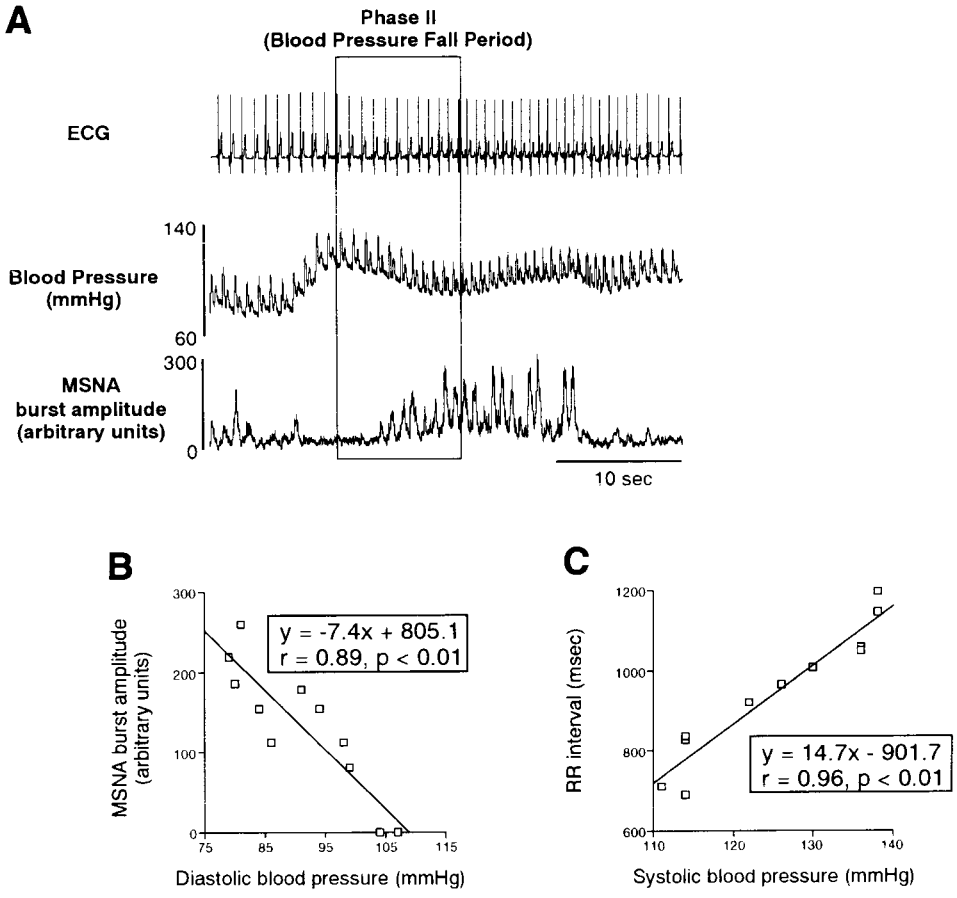

Fig. 1. Simultaneous recordings of electrocardiograms (ECG), blood pressure wave, and integrated muscle sympathetic nerve activity (MSNA) trace (*A*), linear regressions between diastolic blood pressure and MSNA (*B*) and those between systolic blood pressure and R-R interval (*C*) during Valsalva maneuver before head-down bed rest (HDBR) in a subject. Abscissa indicates diastolic (in *B*) and systolic (in *C*) blood pressures, and ordinate represents MSNA burst amplitude (in *B*) and heart rate (in *C*). Regressions were calculated from MSNA burst amplitudes (in *B*) and R-R interval (in *C*) at each blood pressure level. Linear correlations were observed in both trials. Linear equation is $y = -7.4x + 805.1$ ($r = 0.89$, $P < 0.01$) for MSNA (in *B*) and is $y = 14.7x - 901.7$ ($r = 0.96$, $P < 0.01$) for R-R interval (in *C*). Slopes of regression line were defined as baroreflex slopes for MSNA and R-R interval.

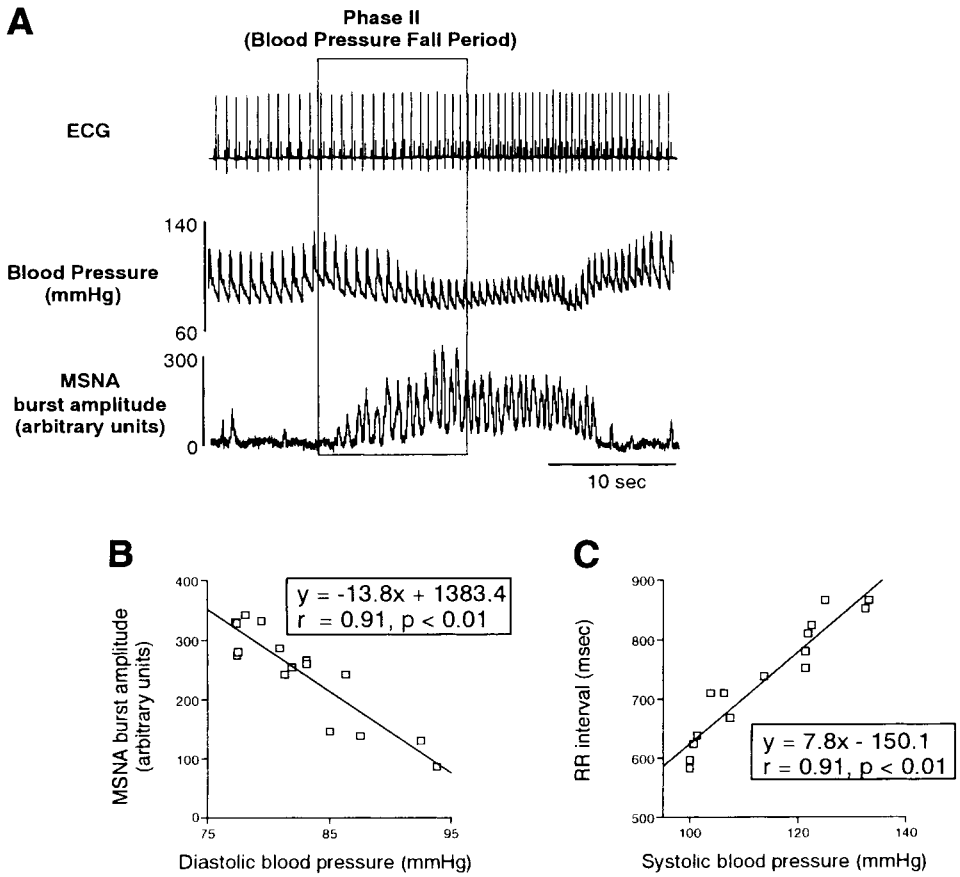

Fig. 2. Simultaneous recordings of variables (*A*), linear regressions between diastolic blood pressure and MSNA (*B*), and those between systolic blood pressure and R-R interval (*C*) during Valsalva maneuver after 120 days of HDBR in same subject as Fig. 1. Abscissa, ordinate, and calculations for regressions are similar to Fig. 1. Linear correlations were observed in both trials. Linear equation is $y = -13.8x + 1{,}383.4$ ($r = 0.91$, $P < 0.01$) for MSNA (*B*) and is $y = 7.8x - 150.1$ ($r = 0.91$, $P < 0.01$) for R-R interval (*C*). Slopes of regression line were defined as baroreflex slopes for MSNA and R-R interval.

II of Valsalva maneuver in supine position on Pre-HDBR and $HDBR_{120}$, respectively, in a typical subject. The baroreflex slopes for MSNA, which were determined as the slopes between diastolic blood preesure and MSNA burst amplitudes, were significantly steeper on $HDBR_{60}$ and $HDBR_{120}$ compared with those on Pre-HDBR. In contrast, the baroreflex slopes for R-R interval, which were determined as the slopes between systolic blood preesure and R-R intervals, were significantly flatter on $HDBR_{60}$ and $HDBR_{120}$ compared with those on Pre-HDBR (Fig. 3). There was no significant difference in blood pressure responses during early phase II of Valsalva maneuver among Pre-HDBR, $HDBR_{60}$, and $HDBR_{120}$.

HUT test. We did not observe any presyncopal symptoms, severe drop in blood pressure, or severe bradycardia that warranted termination of HUT tests in any trials.

MSNA burst rate and total MSNA increased in all HUT tests (Table 2). The increments in total MSNA

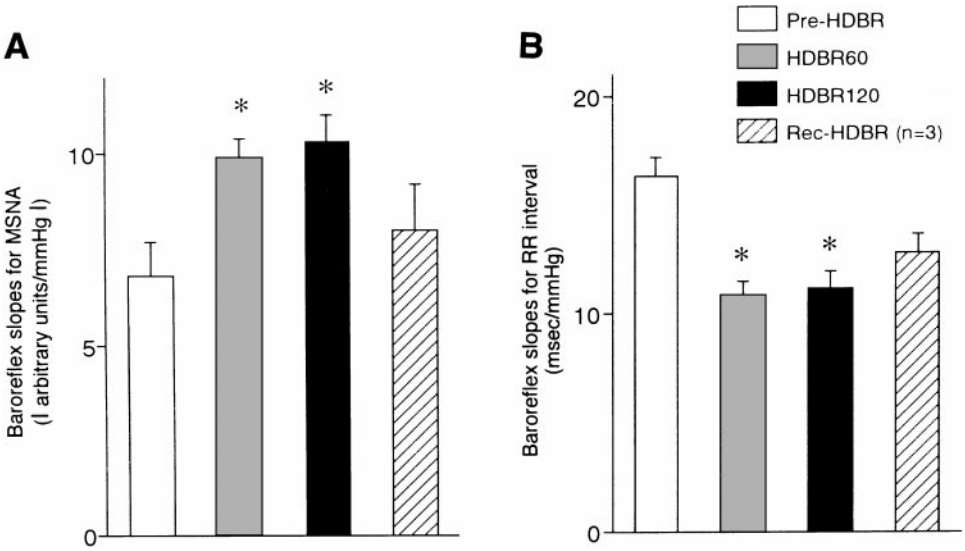

Fig. 3. Baroreflex slopes for MSNA (*A*) and R-R interval (*B*) in Valsalva maneuver before (Pre-HDBR), after 60 ($HDBR_{60}$) and 120 days ($HDBR_{120}$) of HDBR, and during recovery (Rec-HDBR). For Pre-HDBR, $HDBR_{60}$, and $HDBR_{120}$, $n = 6$; for Rec-HDBR, $n = 3$. Values are means ± SE; $^*P < 0.05$ vs. Pre-HDBR. We did not perform statistical analysis of data on Rec-HDBR due to small sample number.

Table 2. *MSNA in HUT tests*

	Supine (0°)	30° HUT	60° HUT
MSNA burst rate, bursts/min			
Pre-HDBR	19.1 ± 2.3	31.0 ± 2.1*	37.3 ± 1.7*
$HDBR_{60}$	29.4 ± 4.2	43.2 ± 4.2*	57.7 ± 6.1*
$HDBR_{120}$	28.3 ± 2.2	41.8 ± 2.2*	55.5 ± 2.0*
Rec-HDBR	23.0 ± 3.3	39.7 ± 5.3	48.5 ± 4.0
Total MSNA, arbitrary units/min			
Pre-HDBR	1,859 ± 279	3,387 ± 330*	4,353 ± 324*
$HDBR_{60}$	2,869 ± 319	5,019 ± 549*	6,784 ± 490*
$HDBR_{120}$	2,456 ± 253	4,530 ± 320*	6,473 ± 536*
Rec-HDBR	2,277 ± 261	4,618 ± 631	5,830 ± 426

MSNA expressed as MSNA burst rate and total MSNA in head-up tilt (HUT) tests at Pre-HDBR, after $HDBR_{60}$ and $HDBR_{120}$, and during Rec-HDBR. For Pre-HDBR, $HDBR_{60}$ and $HDBR_{120}$, $n = 6$; for Rec-HDBR $n = 3$. Values are means ± SE. *$P < 0.05$ vs. supine on each day. We did not perform statistical analysis of data on Rec-HDBR due to small sample number.

Table 3. *Heart rate and mean blood pressure in HUT tests*

	Supine (0°)	30° HUT	60° HUT
Heart rate, beats/min			
Pre-HDBR	65.1 ± 2.3	73.6 ± 2.5*	84.3 ± 2.9*
$HDBR_{60}$	73.0 ± 2.2	83.8 ± 3.8*	95.2 ± 4.0*
$HDBR_{120}$	72.1 ± 2.9	83.7 ± 5.1*	98.6 ± 3.3*
Rec-HDBR	67.0 ± 2.9	75.9 ± 3.3	94.8 ± 2.7
Mean blood pressure, mmHg			
Pre-HDBR	86.3 ± 2.2	87.6 ± 2.5	87.9 ± 2.4
$HDBR_{60}$	89.1 ± 2.3	88.4 ± 1.6	80.3 ± 2.5*
$HDBR_{120}$	89.4 ± 2.2	88.4 ± 2.5	79.6 ± 2.3*
Rec-HDBR	89.8 ± 1.1	88.5 ± 1.8	89.6 ± 2.1

Heart rate and mean blood pressure in HUT tests at Pre-HDBR, after $HDBR_{60}$ and $HDBR_{120}$, and during Rec-HDBR. For Pre-HDBR, $HDBR_{60}$, and $HDBR_{120}$, $n = 6$; for Rec-HDBR $n = 3$. Values are means ± SE. *$P < 0.05$ vs. supine on each day. We did not perform statistical analysis of data on Rec-HDBR due to small sample number.

from supine to 60° HUT on $HDBR_{60}$ and $HDBR_{120}$ were similar to those on Pre-HDBR ($P = 0.05$; Fig 4).

Heart rate increased in all HUT tests (Table 3). The increments in heart rate from supine to 60° HUT were significantly greater on $HDBR_{60}$ and $HDBR_{120}$ (25.2 ± 2.8 and 24.9 ± 4.1 beats/min, respectively) than on Pre-HDBR day (19.1 ± 1.5 beats/min; Fig 4). Mean blood pressure did not change in the HUT tests on Pre-HDBR but decreased on $HDBR_{60}$ and $HDBR_{120}$ (Table 3). Changes in mean blood pressure from supine to 60° HUT were significantly greater on $HDBR_{60}$ and $HDBR_{120}$ (−8.8 ± 1.2 and −9.8 ± 1.3 mmHg, respectively) than those on Pre-HDBR (2.6 ± 0.4 mmHg; Fig 4).

The baroreflex slopes for MSNA in supine (0°) and 60° HUT positions were significantly steeper on $HDBR_{60}$ and $HDBR_{120}$ compared with those of Pre-HDBR. In contrast, the baroreflex slopes for heart rate in supine (0°) and 60° HUT positions were significantly flatter on $HDBR_{60}$ and $HDBR_{120}$ compared with those of Pre-HDBR day (Fig. 5).

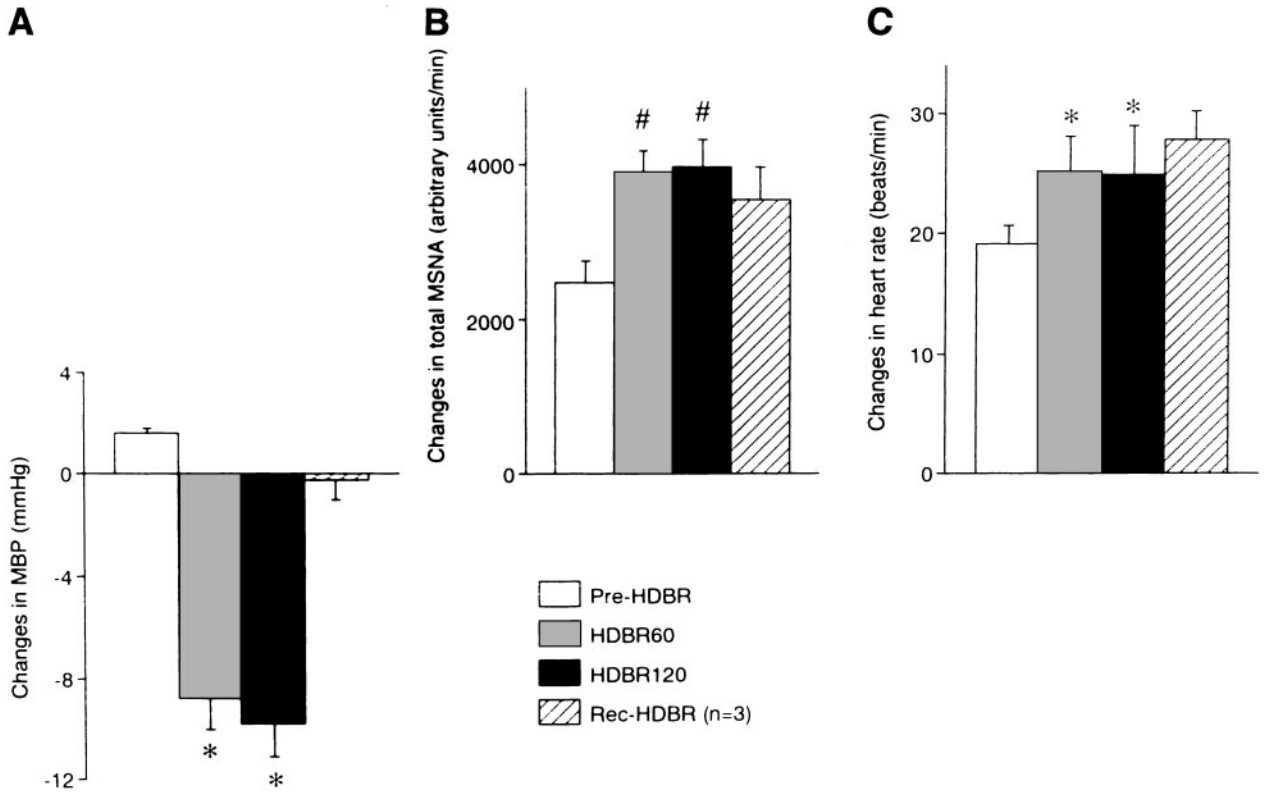

Fig. 4. Changes in mean blood pressure (MBP; *A*), MSNA (*B*), and heart rate (*C*) in response to head-up tilt (HUT) tests at Pre-HDBR, $HDBR_{60}$ and $HDBR_{120}$, and during Rec-HDBR. Values are means ± SE; *$P < 0.05$ and #$P < 0.1$ vs. Pre-HDBR. We did not perform statistical analysis of data on Rec-HDBR due to small sample number.

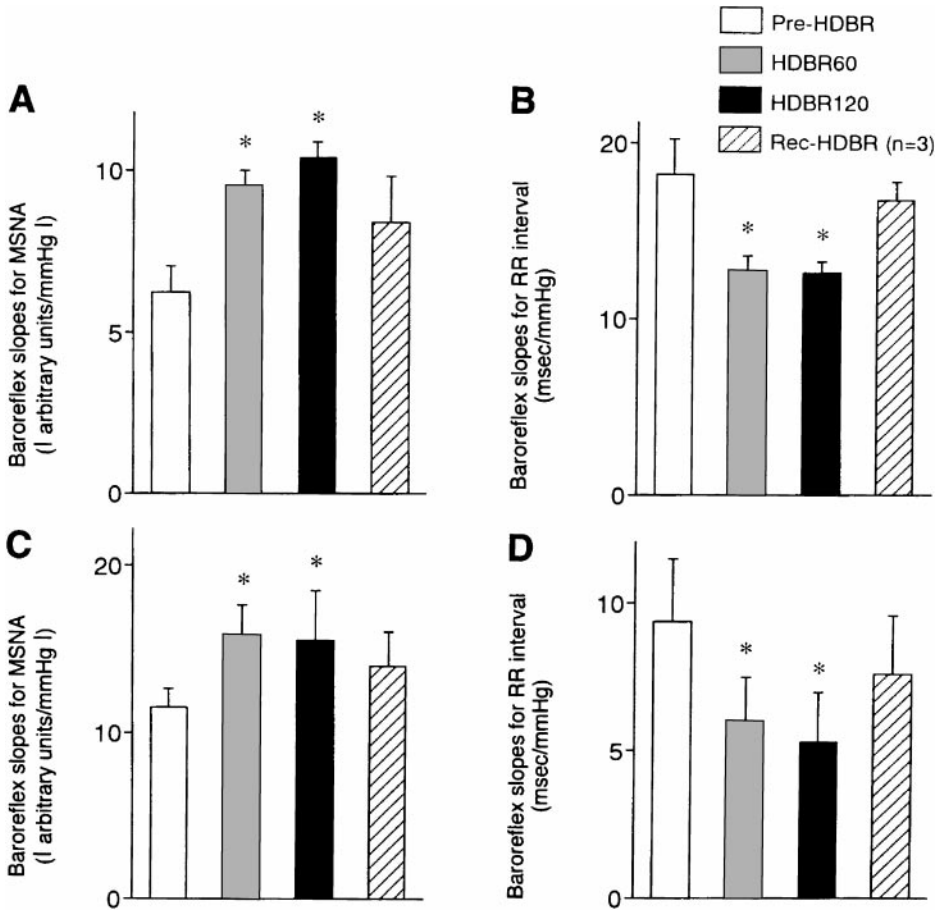

Fig. 5. Baroreflex slopes for MSNA in supine (0°; *A*) and 60° HUT (*C*) position and those for R-R interval in supine (0°; *B*) and 60° HUT (*D*) positions during HUT tests at Pre-HDBR, $HDBR_{60}$ and $HDBR_{120}$, and during Rec-HDBR. For Pre-HDBR, $HDBR_{60}$, and $HDBR_{120}$, $n = 6$; for Rec-HDBR, $n = 3$. Values are means ± SE $*P < 0.05$ vs. Pre-HDBR. We did not perform statistical analysis of data on Rec-HDBR due to small sample number.

DISCUSSION

The important findings of the present study were *1*) resting supine MSNA increased after 60 and 120 days of HDBR, *2*) the baroreflex slopes for MSNA were augmented after HDBR, in contrast to the attenuated baroreflex control of heart rate, and *3*) MSNA increments in response to HUT after HDBR were similar to those before HDBR, but mean blood pressure in 60° HUT decreased after HDBR. The attenuation of baroreflex control of R-R interval after simulated microgravity was consistent with earlier studies (10, 15–17, 19). We emphasize that it may be difficult to manage and reproduce the present experiments due to the long HDBR duration.

In relation to orthostatic hypotension, alterations in peripheral vascular regulation were demonstrated after spaceflight (6, 18, 34). We hypothesized that the baroreflex modulation of vasomotor sympathetic nerve activity, which controls peripheral vascular resistance, may be impaired, and may thus cause a reduction in vasomotor sympathoexcitation, contributing to orthostatic hypotension after exposure to simulated microgravity. Consequently, we compared the baroreflex slopes for MSNA during early phase II of Valsalva maneuver (blood pressure fall period) and in supine and 60° HUT positions after long-term HDBR to those before HDBR. However, the baroreflex slopes for MSNA were significantly steeper on $HDBR_{60}$ and $HDBR_{120}$ than on Pre-HDBR under all experimental conditions, demonstrating that the baroreflex control of MSNA was not attenuated, but augmented after 60 and 120 days of simulated microgravity. In addition, increases in MSNA in response to HUT were maintained well after long-term HDBR, whereas mean blood pressure decreased in 60° HUT position after HDBR. Therefore, our results suggest that neither impairment of baroreflex control of vasomotor sympathetic outflow to skeletal muscle nor attenuation of vasomotor sympathoexcitation in response to orthostasis are contributing factors in orthostatic hypotension after long-term simulated microgravity.

The mechanisms of augmentation in baroreflex control of MSNA after long-term HDBR are unclear. They may include changes in afferentation from arterial and/or cardiopulmonary baroreceptors in association with changes in central processing. Another possibility includes effects of humoral factors and those of altered afferent inputs from affected organs: skeletal muscle (37) and heart (23). In addition, long-term HDBR might reduce a d*P*/d*t* of the systolic pressure rise in association with a reduced cardiac inotropy. A dog experiment (8) showed that a shift from static to pulsatile pressure increased carotid baroreceptor afferent nerve activity; this effect was attributed to the responsiveness of the receptors to positive d*P*/d*t*. Therefore, a reduced d*P*/d*t* after long-term HDBR could decrease baroreflex inhibi-

tion, increase sympathetic activity, and, consequently, induce a steeper baroreflex slope for MSNA after 60 and 120 days of HDBR.

Moffitt et al. (26) documented that arterial baroreflex modulation of sympathetic nerve activity was attenuated after 14 days of hindlimb unloading in rodents. The discrepancy between their findings and ours could be attributed to the different species used (upright humans vs. quadripedal rats), invasiveness (including surgical procedures and anesthesia) of methods, microgravity-simulating models (6° HDBR vs. hindlimb unloading), and exposure duration (120 days vs.14 days).

After long-term HDBR, the increase in MSNA from supine to 60° HUT was similar to the pre-HDBR level despite the augmented reflex slopes for MSNA in response to blood pressure fluctuations in 60° HUT position. One possible explanation for this is changes in cardiopulmonary baroreflex control of MSNA after long-term HDBR. Loading of cardiopulmonary baroreceptors may be more important in causing peripheral vasoconstriction than sinoaortic baroreceptors (7). Convertino et al. (11) reported that 7 days of HDBR increased the gain of the cardiopulmonary baroreflex control of forearm vascular resistance in humans during simulated orthostasis. Hypovolemia, which commonly occurs during and after HDBR, may reduce cardiopulmonary baroreflex inhibition of sympathetic vasoconstrictive drive. However, chronic and long-term loading of cardiopulmonary baroreceptors due to central fluid expansion induced by HDBR could change central modulations of cardiopulmonary afferents and their interactions with the arterial baroreflex function. Thus it remains unclear how long-term HDBR affects cardiopulmonary baroreflex control of sympathetic vasoconstriction. Another possible explanation is alterations in afferent inputs from other systems, i.e., vestibular system and antigravity muscles, which could tentatively affect the contribution of arterial baroreflex function to the magnitude of vasomotor sympathoexcitation after long-term HDBR, because multiple systems can contribute to maintaining upright posture. However, it remains unclear how augmentation of arterial baroreflex control of MSNA affects the magnitude of vasomotor sympathoexcitation under orthostasis after long-term HDBR.

Our findings showed that mean blood pressure fell at 60° HUT after 120 days of HDBR, though incremental responses of MSNA and heart rate were maintained. The mechanisms of the decrease in mean blood pressure in HUT tests after HDBR are unknown, but there are several possible explanations. First, the regulation of sympathetic outflow could vary for different vascular beds. There was a difference in vasomotor sympathetic controls between forearm and leg under applied stimuli (1, 28) and between viscera and skeletal muscle after simulated microgravity (35, 36). The rate of norepinephrine depletion in the spleen was attenuated in rodents during exercise after hindlimb unloading associated with increases in blood flow to the spleen and kidney, in contrast to enhanced vasoconstrictive responses in skeletal muscle (35, 36). However, it was reported that there were positive correlations between MSNA and norepinephrine spillover in the heart (32) and kidney (33), suggesting that MSNA reflects sympathetic vasoconstrictor traffic to at least three vascular beds controlling a large part of total peripheral resistance. Second, the exposure to simulated microgravity could produce a reduction in the release of norepinephrine from sympathetic nerve terminals, which was indicated by Goldstein et al. (20). Third, adaptation to simulated microgravity might induce decreased sensitivity of vessels to sympathetic vasoconstrictor influences through either alterations in adrenoreceptor sensitivity of vessels, or in the sensitivity of a given vascular wall to constrictor influences. However, recent work demonstrated that HDBR had little effect on the vasoconstrictive response to α-adrenergic stimulation (12). Fourth, muscle pump could become ineffective because of muscle atrophy after HDBR (27). We observed severe atrophy of the leg muscles, particularly in antigravity muscles. It was documented that the maximal strength of the triceps surae muscle was reduced by 36.7% after the same HDBR (37). The muscle atrophy could depress intramuscular pressure during HUT tests after HDBR, resulting in ineffective muscle pump. Other possibilities are interactions with vasodilators, such as nitric oxide, and increments in venous compliance (9, 24).

Our findings showed that resting supine MSNA increased after 60 and 120 days of HDBR. One possible mechanism for vasomotor sympathoexcitation after long-term simulated microgravity is that HDBR-induced circulatory blood volume loss might elicit an enhancement of sympathetic nerve activity through baroreflex function. Other mechanisms may be connected with peripheral changes: increased venous compliance (9, 24), decreased muscle stiffness, and muscle atrophy (37). Changes in afferentation from effected muscles may be involved. Our findings were inconsistent with a previous work by Shoemaker et al. (30), demonstrating that MSNA was reduced after 14 days of HDBR. We cannot explain the difference in results, but there is a possibility that the duration of the exposure to HDBR may partly affect resting supine levels of MSNA after simulated microgravity.

In conclusion, the baroreflex control of vasomotor sympathetic nerve activity to skeletal muscle was augmented, while the same reflex control of heart rate was attenuated after 120 days of HDBR.

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