Simulated microgravity produces attenuated baroreflex-mediated pressor, chronotropic, and inotropic responses in mice

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METHODS

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Part 1: Common Protocol

After halothane (3%) induction in an anesthetic chamber, mice were anesthetized using a dedicated vaporizer with 1.5% halothane added to a constant flow of 1:1 O2-N2, which was delivered using an adapted face mask. Anesthesia was maintained with halothane during the entire surgery, and 1:4 albumin-saline was administered via the right jugular vein with a 30-gauge needle. The cervical region was exposed via a midline ventral incision, the right and left thyroid lobes were retracted, and the sternomastoid muscles were retracted bilaterally. The sternohyoid was looped with 6-0 silk and retracted over the trachea to expose the common carotid sinus and artery region. Carotid arteries parallel to the trachea were carefully isolated. After 5 min of steady-state MAP and HR monitoring and measurement, a baroreflex response was induced by selective carotid baroreceptor unloading through transient occlusion of both carotid arteries (TBCO) with microclips (Accurate Surgical and Scientific Instruments, Westbury, NY). After 10–15 s of TBCO, MAP and HR were measured. Microclips were released, and steady-state MAP and HR readings were maintained before another trial was attempted. This was repeated four to six times in each mouse.

The afferent neural signal was generated by a change in blood pressure sensed by the carotid baroreceptors, distal to the point of carotid occlusion. However, other baroreceptors in the aortic arch and heart that lie proximal to the point of occlusion will not sense a decrease in arterial pressure and, thus, will buffer the cardiovascular effector responses. Hence, there is a need for aortic arch baroreceptor deafferentation. The relative locations of the aortic depressor nerve (ADN) and the sympathetic trunk (ST), the two major buffering afferents, have been previously reported (24).

Groups of mice. One group of mice acted as controls (n = 9), in which the above-described protocol was performed with intact ADN and ST. A second subgroup of mice acted as denervated controls (DC, n = 7). In these mice, afferent signals from the aortic arch and cardiopulmonary baroreceptors were eliminated by bilateral sectioning of the ADN and ST (25), allowing for complete isolation of the carotid baroreceptor reflex.

After denervation of the ADN and ST, the ganglionic blocker hexamethonium bromide (10 μg/kg in 200 μl of saline) was administered to a third subgroup of mice (Hex, n = 4). Hexamethonium was administered via the right jugular venous line, and 5 min were allowed for effect before any procedures were done.

A fourth group of mice (HLU, n = 6) was subjected to HLU for 14–21 days before physiological assessment. HLU mice were briefly anesthetized with halothane to minimize discomfort to the animal during the process. The tail was cleaned, and a light coat of tincture of benzoin was applied. The tail was air-dried until it was tacky. The adhesive strip tapes were looped through a swivel harness and applied to a freely rotating ball-bearing line, allowing free 360° rotation. The hindlimb of the mouse was elevated to create a 35° angle with the ground, so that only the front limbs were in contact with the floor. After the specified duration of HLU, the mice were lowered, weighed, and subjected to cardiovascular parameters measured in response to TBCO with ADN and ST denervation. All animals in the HLU group were denervated before physiological assessment to provide unbuffered comparison with DC mice.

In all subgroups of mice mentioned above, MAP and HR were measured before, during, and after TBCO, as described above. MAP was measured via a femoral arterial line (Tapered R-FAC microcem-nath tube, Brain Tree Scientific, Braintree, MA) linked to a pressure transducer (Statham P23 Db), and HR was obtained from the blood pressure trace via a Biotach/ECG transducer. The data were collected at 1,000 Hz (Biopac Data Acquisition, Biopac Systems, Santa Barbara, CA) and digitally stored.

Part 2: Measurement of In Vivo Myocardial Contractile Responses (Pressure-Volume Loops)

In a study separate from part 1, a different group of mice was subjected to HLU for 14–21 days (n = 6) as described above and were compared with caged control mice that were not subjected to HLU (DC, n = 20). None of the mice in this study were used in part 1 or part 3. Mice were anesthetized with a combination cocktail of morphine (80 μg/kg), urethane (200 μg/kg), and etomidate (120 μg/kg) and were ventilated (inspired O2 fraction = 100%, tidal volume = 200 μl) with a sinusoidal solenoid valve after tracheotomy. Buffering nerves (ADN and ST) were denervated, and carotid arteries were isolated as described above. After a subternal lateral thoracotomy, a 1.4-Fr micromanometer-conductance catheter (Millar Instruments, Houston, TX) was advanced retrogradely into the LV by an apical stab wound made with a 30-gauge needle through the longitudinal cardiac axis, as previously described (4, 38). The catheter was advanced until the distal tip was placed in the aortic root and the proximal electrode just within the endocardial wall of the LV apex. Albumin-saline (1:4) was administered via the right jugular vein with a 30-gauge needle. Offset calibration of the recorded volume signal was obtained by a saline-washin technique, and stroke volume calibration was derived from direct measurement of aortic blood flow, obtained by using a flow probe (model AT01RB, Transonics, Ithaca, NY) (4, 38). Pressure, volume, and flow signals were digitized at 1 kHz, stored on a disk, and analyzed with custom software.

Indexes of myocardial systolic and diastolic performance were derived from pressure-volume data obtained at steady state and during transient unloading via vena caval occlusion (VCO) of the heart. Baseline and VCO readings were acquired before and after TBCO for HLU and DC mice.

VCO data were used to determine the slope of the end-systolic pressure-volume relation (ESPVR) and the first derivative of the pressure (dP/dt)-end-diastolic volume relation. Myocardial contractility was indexed by the peak rate of rise in LV pressure (dP/dt) divided by instantaneous pressure and the load-independent end-diastolic elastance (Eso), i.e., the slope of the ESPVR. Although the ESPVR is nonlinear in mice, over the range of data obtained by preload and afterload changes, the ESPVR can be considered linear (21). End systole is measured as the point of peak elastance [peak pressure-to-volume (P-V) ratio] and are plotted for each P-V loop. A linear regression line is fit through these points, and the slope is derived. Over the range of the data obtained by VCO, the ESPVR was found to be linear with minimal error. Baseline cardiac preload was indexed as the LV end-diastolic volume and end-diastolic pressure. Cardiac afterload was evaluated with effective arterial elastance (Ea, i.e., the ratio of LV systolic pressure to stroke volume).

Part 3: In Vitro Myocyte Studies

Mice for this in vitro study were not used for the in vivo studies mentioned above. Myocytes (n = 27 for each group) from HLU (n = 3) and age-matched control mice (control, n = 3) were isolated. Hearts were perfused with Ca2+-free buffer containing (in mmol/l) 120 NaCl, 5.4 KCl, 1.2 MgSO4, 1.2 NaH2PO4, 5.6 glucose, 20 NaHCO3, 10 2,3-butanedione monoxime (Sigma), and 5 taurine (Sigma), gassed with 95% O2-5% CO2, and then subjected to enzymatic digestion with collagenase type 2 (1 mg/ml; Worthington) and protease type XIV (0.1 mg/ml; Sigma). Myocytes were obtained by mechanical disruption of digested hearts, filtration, centrifugation, and resuspension in 0.125 mmol/l Ca2+-Tyrode solution containing (in mmol/l) 144 NaCl, 1 MgCl2, 10 HEPES, 5.6 glucose, and 5 KCl, with pH adjusted to 7.4 with NaOH. Myocytes were resuspended in 0.25 mmol/l Ca2+-Tyrode solution and then in 0.5 mmol/l Ca2+-Tyrode solution and stored in Tyrode solution containing 0.5 mmol/l probenecid and 1.8 mmol/l Ca2+.

Myocytes were incubated with 5 μmol/l fura 2-AM (Molecular Probes, Eugene, OR) and then transferred to a Lucite chamber on the
stage of an inverted microscope (model TE 200, Nikon) and continuously superfused with Tyrode solution containing 1.8 mmol/l Ca$^{2+}$ and 0.5 mmol/l probenecid. Sarcomere length and Ca$^{2+}$ transients were measured in myocytes stimulated with increasing doses of isoproterenol. These experiments were conducted in room air (Po$_2$ ~21%).

Sarcomere length was recorded with a charge-coupled device camera (model iCCD, IonOptix). Change in average sarcomere length was determined by fast Fourier transform of the Z-line density trace to the frequency domain. Intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) was measured using the Ca$^{2+}$-sensitive dye fura 2 and a dual-excitation spectrofluorometer (IonOptix, Milton, MA) alternately excited with a xenon lamp at wavelengths of 365 and 380 nm. The emission fluorescence was reflected through a barrier filter (510 nm) to a photomultiplier tube. The fura 2 fluorescence ratio, i.e., the ratio of the photon live count detected by excitation at 365 nm to that detected by excitation at 380 nm, represents [Ca$^{2+}$].

Statistics
Statistical analyses were performed using StatView (SAS Institute). Values are means ± SE unless otherwise stated. The differences between each group were determined using ANOVA. Post hoc analyses were performed by using Student-Newman-Keuls test. $P < 0.05$ was considered significant.

RESULTS

Part 1

HR and blood pressure responses to TBCO. Baseline MAP and HR were similar in control and DC mice (Table 1). As expected, TBCO increased MAP and HR in control mice, whereas the TBCO-stimulated increase in MAP and HR was substantially enhanced in DC compared with control mice ($\Delta$MAP = 40 ± 2 vs. 17 ± 2 mmHg, $\Delta$HR = 33 ± 3 vs. 15 ± 2 beats/min, n = 9, $P < 0.01$ for both; Fig. 1A, Table 1). Representative HR and MAP traces are shown in Fig. 1B for control and DC mice. The carotid sinus baroreflex-mediated HR and MAP increases are substantially enhanced after ADN and ST deafferentation, consistent with the concept that these nerve trunks were buffering the response to baroreceptor unloading of the TBCO.

To confirm that the changes in MAP and HR are mediated by the autonomic nervous system and are not solely due to the mechanical impact of TBCO, animals were subjected to TBCO after ganglionic blockade with hexamethonium (10 $\mu$g/kg). Hexamethonium caused marked reductions in HR and MAP baroreflex responses ($\Delta$MAP = 8 ± 2 mmHg, $\Delta$HR = 9 ± 1 beats/min, n = 4, $P < 0.001$ vs. DC; Table 1, Fig. 1A).

Table 1. Part 1: HR and MAP at baseline and TBCO

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Occlusion</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>86 ± 3</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>DC</td>
<td>7</td>
<td>80 ± 3</td>
<td>119 ± 3</td>
</tr>
<tr>
<td>Hex</td>
<td>4</td>
<td>75 ± 4*</td>
<td>83 ± 5‡</td>
</tr>
<tr>
<td>HLU</td>
<td>6</td>
<td>82 ± 3</td>
<td>96 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of mice. C, control (nondenervated); DC, denervated control; Hex, hexamethonium; HLU, hindlimb unweighting; MAP, mean arterial pressure; HR, heart rate; TBCO, transient bilateral carotid occlusion. *Significantly different from C ($P < 0.01$). †Significantly different from Hex ($P < 0.01$). ‡Significantly different from HLU ($P < 0.01$). §Significantly different from all other groups ($P < 0.01$).

Fig. 1. Impact of transient bilateral carotid occlusion (TBCO) on heart rate (HR) and mean arterial pressure (MAP). A: responses in control nondenervated (C) mice ($n = 9$), mice denervated by sectioning of the aortic depressor nerve and sympathetic trunk (DC, $n = 7$), and mice denervated and treated with the ganglionic blocker hexamethonium (Hex, $n = 4$). TBCO stimulates increases in HR and MAP. Denervation of buffering nerves potentiates MAP and HR responses to TBCO. *$P < 0.001$ vs. DC. Hex obliterated HR and MAP responses to bilateral carotid artery occlusion. *$P < 0.001$ vs. DC. Values are ± SE. B: representative examples of blood pressure and HR (bpm, beats/min) traces for DC and control (C) groups before, during, and after TBCO. DC responses to TBCO are augmented compared with control.
Impact of HLU on baroreflex function. In all HLU mice, buffering nerves were denervated before TBCO. Baseline HR and MAP were similar in HLU and DC mice. However, in HLU mice, HR and MAP responses to TBCO were markedly attenuated: \( \Delta \text{MAP} = 14 \pm 1 \text{ mmHg} \) and \( \Delta \text{HR} = 10 \pm 2 \text{ beats/min} \) \((n = 6, P < 0.01 \text{ vs. DC; Table 1, Fig. 2})\).

Part 2: Integrated Cardiovascular Responses and Myocardial Contractility

Table 2 summarizes heart weight-to-body weight ratios as well as baseline hemodynamic data from DC (all animals undergoing integrated cardiovascular interrogation were denervated) and HLU mice. The mice were instrumented with a micromanometer-conductance catheter. HR and systolic pressure were lower in HLU than in DC mice (Table 2). Similarly, baseline indexes of myocardial contractility were lower in HLU than in DC mice. Furthermore, there was a significant decrease in the heart weight-to-body weight ratio in HLU mice, suggesting a component of myocardial atrophy in this model (Table 2).

The inotropic and pressor response to TBCO was also markedly attenuated in HLU (Table 2). Increases in ventricular elastance (\( E_{es} \)) and \( E_{a} \) were greatly reduced in HLU compared with DC mice: \( E_{es} = 86 \pm 13\% \) vs. 14 ± 10 and \( E_{a} = 38.8 \pm 8 \% \) vs. 8.1 ± 3\% \((n = 6–10, P < 0.05)\). Figure 3 depicts representative P-V loops during VCO before and after TBCO. TBCO increased the slope of the ESPVR (\( E_{es} \)), consistent with a positive inotropic response, and this response was markedly attenuated in HLU (Fig. 3). There were no significant differences in preload indexes between HLU and DC mice at baseline and after TBCO (Table 2).

Part 3: Isolated Myocyte Studies

The demonstration that activation of the endogenous sympathetic response resulted in not only a markedly attenuated pressor response but also a depressed contractility response prompted us to study the contractile reserve in isolated cardiac myocytes from HLU and control mice. Myocytes were isolated from a separate group of control and HLU mice. Simultaneous sarcomere shortening and \( [\text{Ca}^{2+}] \), were measured in isolated myocytes in response to increasing concentrations of the \( \beta \)-agonist isoproterenol. Isoproterenol increased sarcomere shortening in a dose-dependent manner in control and HLU mice (Fig. 4). However, the contractile response to isoproterenol was significantly attenuated in the HLU mice (Fig. 4). Although the percent change in sarcomere shortening was significantly depressed in HLU mice, there was no significant difference in \( \text{Ca}^{2+} \) transients in response to \( \beta \)-adrenergic receptor activation in control and HLU mice. This suggests that the mechanism responsible for depressed contractility in HLU mice involves

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**Table 2. Part 2: Baseline and percent change in hemodynamic parameters**

<table>
<thead>
<tr>
<th></th>
<th>DC</th>
<th>HLU</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>20</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>664±8</td>
<td>590±14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>28±3</td>
<td>26±4</td>
<td>0.3</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>0.14±0.02</td>
<td>0.11±0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Preload</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>8±2</td>
<td>6±2</td>
<td>0.4</td>
</tr>
<tr>
<td>EDV, ml</td>
<td>26±1</td>
<td>25±1</td>
<td>0.6</td>
</tr>
<tr>
<td>Afterload</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>119±2</td>
<td>102±6</td>
<td>0.002</td>
</tr>
<tr>
<td>Contractility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt, mmHg/s</td>
<td>17,242±705</td>
<td>12,667±360</td>
<td>0.002</td>
</tr>
<tr>
<td>PRSW, mmHg/ml</td>
<td>224±130</td>
<td>78±3</td>
<td>0.02</td>
</tr>
<tr>
<td>dP/dt/EDV, mmHg/s(^{-1}) ml(^{-1})</td>
<td>991±76</td>
<td>560±135</td>
<td>0.03</td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>38±3</td>
<td>11±6</td>
<td>0.0001</td>
</tr>
<tr>
<td>( E_{es} ), mmHg/ml</td>
<td>38±8</td>
<td>8.1±3</td>
<td>0.03</td>
</tr>
<tr>
<td>Contractility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( E_{es} ), mmHg/ml</td>
<td>86±13</td>
<td>14±10</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), number of mice. EDP and EDV, end-diastolic pressure and volume; SBP, systolic blood pressure; PRSW, preload-re- cruitable stroke work relation; dP/dt, 1st derivative of pressure; \( E_{es} \) and \( E_{a} \), arterial and end-systolic elastance, respectively. All comparisons considered significant at \( P < 0.05 \).
alterations in Ca\textsuperscript{2+}/H\textsubscript{11001} sensitivity or decreased myocardial mass, rather than alterations in [Ca\textsuperscript{2+}/H\textsubscript{11001}].

DISCUSSION

The purpose of this study was to examine the independent contributions of pressor, chronotropic, and inotropic responses to carotid baroreflex activation, with the goal of understanding the specific derangements and contribution to OI after microgravity. To accomplish this, we utilized a physiological, rather than a pharmacological, stimulus to unload carotid baroreceptors and demonstrated significant influences on all three cardiovascular responses: MAP, HR, and myocardial contractility. Importantly, all three responses due to TBCO were markedly impaired in HLU. Thus all three of these cardiovascular responses may be considered potential targets for interventions designed to treat OI.

A major new finding of this study is that myocardial contractility is clearly augmented in response to TBCO and that this effect is profoundly impaired by HLU. Data obtained in space and in ground-based human and animal studies have been at odds with regard to cardiac contractile impairment in microgravity-associated OI. Koenig et al. (23) demonstrated reduced cardiac output and MAP during lower body negative pressure associated with lowered peak dP/dt in rhesus monkeys exposed to head-down tilt for 4 days. On the other hand, Ray et al. (33) demonstrated no change in baseline dP/dt in...
HLU rats. Our study using isovolumic phase (peak +dP/dr) and end-ejection (Ees) indexes of myocardial contractility and isolated myocyte studies suggest that HLU leads to reduced baseline and baroreflex-stimulated LV contraction. These findings cannot be attributed solely to reduced pressor responses (afterload), inasmuch as we confirmed reduced contractility with the preload- and afterload-independent index Ees. In addition, isolated myocytes demonstrate impaired contractile responses to β-adrenergic receptor activation (reduced contractile reserve). The observation that depressed sarcomere shortening is associated with no alteration in [Ca^{2+}], suggests a mechanism other than that involving dysregulation of Ca^{2+} cycling but, perhaps, an alteration of contractile sensitivity or mechanical alterations in the contractile apparatus itself. This is consistent with the finding that cardiac mass as a percentage of total body weight is decreased, providing a potential explanation for the depressed contractile reserve without an effect on the Ca^{2+} transient.

Our study supports the hypothesis that the contractile responses of the heart may be involved in OI. Adaptational changes in cardiac structure may have a significant influence on cardiac function and are supported by previous findings in humans and animals. The loss in ventricular mass found in HLU agreed with the echocardiographic measurements from the Skylab 4 mission. Arbeille et al. (2) showed that the LV wall thickness (measured by echocardiography) was significantly reduced in subjects after 6 wk of bed rest. In addition, Levine et al. (24) found that, after 2 wk of bed rest, normal subjects showed a significant reduction in ventricular mass (measured by MRI). Moreover, studies with rats in real or simulated microgravity also showed significant differences with respect to cardiac structure. Goldstein et al. (20) reported that the average cross-sectional area of LV papillary muscle fibers was significantly decreased by 20% in rats flown on COSMOS 2044 for 14 days. Thus the decreased LV mass found in HLU mice correlates well with the cardiac echocardiographic data found in astronauts and the heart weights of rats after HLU. Our findings demonstrating a reduced heart weight-to-body weight ratio as well as a [Ca^{2+}]-independent mechanism explaining impaired contractile reserve are congruent with the hypothesis regarding cardiac atrophy.

Our findings with regard to HR attenuation are consistent with observations in humans after exposure to microgravity and prolonged bed rest. Fritsch-Yelle et al. (18) reported decreased slope, range, and operational point of HR responses to baroreceptor activation (by using a neck pressure device) in astronauts, whereas Kamiya et al. (22) demonstrated similar findings in individuals after prolonged (120 days) bed rest. This is further confirmed and highlighted by the Neurolab findings (8). Although the diminished HR response after HLU in mice agrees with human bed rest and microgravity studies, attenuated HR responses are less obvious in rats. Overton et al. (30) and Tipton (37) examined HR responses to various sympathomimetic agents and lower body negative pressure in HLU rats and found that HLU affected baroreflex pressor, but not HR, responses. Nevertheless, it is possible that this discrepancy may be a function of the difference in methods used to elicit baroreflex responses (lower body negative pressure vs. TBCO) or the duration of HLU (2 days vs. 21 days).

Similarly, diminished pressor responses have also been observed in animal models and in bed rest and postflight studies.
11–13, 15, 26, 34, 39), it remains unclear whether this amount of time represents an appropriate simulation. Furthermore, it remains to be determined whether this represents long- or short-term exposure, given the life span of rodents (~2 yr). Time-response experiments will be necessary to determine the differential effect of duration of HLU on cardiovascular changes.

A further potential limitation is that TBCO represents an extreme form of baroreceptor unloading. However, to our knowledge, there is no technique available to test the blood pressure response to unloading other than this open-loop technique. Our laboratory has previously performed a graded unloading technique by completely isolating the carotid sinus baroreceptor in a rat (36). In this study, the MAP-carotid sinus pressure relation was determined by stepwise changes in the carotid sinus pressure. The average peak gain (ΔP/ΔISP, where P is arterial pressure and ISP is intrasinus pressure) was obtained from the maximum slope (point to point) of the arterial pressure-carotid sinus pressure relation. The peak gain in the rat is ~2.0 and occurs at a carotid sinus pressure of ~110 mmHg. The responses are completely flat at <60 and >160 mmHg. If this can be extrapolated to the mouse, we can surmise that although TBCO may seem to represent an extreme perturbation, the responses suggest that the system is responding over its defined operating range. Thus, even if the carotid sinus pressure is below ~60 mmHg during TBCO, we would not predict any further response.

Indeed, it would be ideal to be able to completely isolate the carotid sinus of the mouse and generate MAP-carotid sinus pressure relations. These experiments, in combination with carotid sinus of the mouse and generate MAP-carotid sinus pressure, provide countermeasures for OI in astronauts and in patients subjected to prolonged bed rest.

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REFERENCES


GRANTS

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CARDIOVASCULAR FUNCTION AND MICROGRAVITY IN MICE


