The mouse as a model of cardiovascular adaptations to microgravity

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Powers, Jennifer, and Daniel Bernstein. The mouse as a model of cardiovascular adaptations to microgravity. J Appl Physiol 97: 1686–1692, 2004. First published July 16, 2004; doi:10.1152/japplphysiol.00925.2003.—There are a multitude of physiological adaptations to microgravity, involving the cardiovascular, neuromuscular, and neuroendocrine systems. Some of these adaptations lead to cardiovascular deconditioning on return to normal gravity, posing a threat to human functional integrity after long-term spaceflight. Animal models of microgravity, e.g., tail suspension in rats, have yielded important information regarding the mechanism of these adaptations and have been useful in the design of countermeasures. The mouse could potentially be a useful experimental model, given its small size (smaller and lighter payload) and the powerful tools of experimental mouse genetics, which allow us to dissect mechanisms on a gene-specific basis. We show that the mouse demonstrates a wide range of cardiovascular responses to simulated microgravity, including alterations in heart rate, exercise capacity, peripheral arterial vasodilatory responsiveness, and baroreflex response. These responses are qualitatively similar to many of those demonstrated in humans during spaceflight and in rats using tail suspension, although there are some important differences. Thus the mouse has value as a model for studies of cardiovascular changes during microgravity; however, investigators must maintain an appreciation of important species differences.

baroreflex; autonomic nervous system

Physiological adaptations to microgravity involve alterations in cardiovascular, neuromuscular, and neuroendocrine systems. These adaptations result in cardiovascular deconditioning and orthostatic hypotension (1, 5) on return to normal gravity, posing a threat to human functional integrity after long-term spaceflight (6). Several mechanisms for this deconditioning have been proposed, including alterations in body fluid composition and abnormalities of sympathetic nervous system function (7, 13, 29). Human studies of cardiovascular function in microgravity are limited by the difficulties of controlling for exogenous factors such as diet, fluid intake, baseline level of physical fitness, baseline level of sympathetic nervous activity, as well as genetic background. This underscores the importance of animal models of microgravity for increasing our understanding of cardiovascular adaptations and for the development of effective countermeasures.

In small mammals, the tail suspension model has been widely used as a model for simulated microgravity (42, 43). Studies, predominantly in rats, have demonstrated that tail suspension has good fidelity to many of the cardiovascular changes that occur in larger mammals during real microgravity exposure. Whereas the rat has been the principal animal utilized in these simulations, there are a number of reasons why the mouse could ultimately be a better model. The mouse is more than an order of magnitude smaller than the rat, resulting in reduction in space requirements, payload weight, and cost. Many laboratories have demonstrated the feasibility of performing complex cardiovascular measurements in the mouse and have shown substantial similarities between the mouse and the human in cardiovascular responses to exercise, pharmacological agents, and alterations in cardiac-loading conditions (11). The mouse is now widely accepted as a reasonable model in which to study human cardiovascular diseases (36). Most importantly, the mouse is the only mammalian species for which it has been routinely possible to target genetic disruptions, producing gene knockout animals. Conditional knockouts are now possible in which gene expression can be selectively turned on and off at will. These methods of experimental mouse genetics give us extraordinarily powerful tools, which allow us to microdissect the contributions of specific control pathways in cardiovascular regulation.

Although there have been a few tail suspension studies in mice (31, 33, 34), to date these have not included detailed studies of cardiovascular adaptations. The purpose of the present study was to examine in detail the cardiovascular response of the mouse to tail suspension and validate the mouse as a model for each of the alterations encountered in humans with microgravity.

Methods

Implantation of Telemetric Units

Twelve-week-old male C57BL6J/DBA mice were instrumented with an implantable telemetric unit (PhysioTel, Data Sciences, St. Paul, MN) allowing for daily noninvasive recordings of the electrocardiogram and calculation of heart rate). Mice were anesthetized with inhaled isoflurane (3%), induced in a closed chamber and maintained via nose cone (1–2%) throughout the surgery. An abdominal midline incision was made on the ventral surface. Two smaller incisions were made in each side of the pectoral region for sutureing the telemetric leads securely to the chest wall. These leads were tunneled subcutaneously and sutured with 5-0 surgical silk suture (Ethicon, Somerville, NJ) into the pectoral muscles. The 3.5-g wireless transmitter was subsequently inserted into the abdominal cavity. To anchor the implant in place, it was sutured with 5-0 surgical silk to the abdominal muscles to ensure limited motion when the mouse was active. Skin incisions were closed with 5-0 surgical silk. The mice were then allowed to recover for 2 wk in a standard rodent cage with food and water available ad libitum before any further studies were performed.

Tail Suspension Protocol

Mice were then subjected to tail suspension by a modification of the method of Morey et al. (24, 25), as detailed in the National Aeronautics and Space Administration Ames Institutional Animal

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Care and Use Committee-approved tail suspension protocol (generously provided to us by Dr. E. Holton). Modifications of this protocol have been used previously in mice to study immune system and skeletal muscle and bone changes with simulated microgravity (31, 33, 34). Normal day-night lighting cycles were maintained, and mice had access to ad libitum water and food. Mice were tail suspended, which we will identify as the “unloaded” state, for a total of 14 days, after which they were returned to the normal four-extremity weight bearing “reloaded” position. Similar numbers of control mice of the same strain background were instrumented and monitored in similar fashion under identical cage conditions but without tail suspension.

Heart Rate Data Acquisition

Electrocardiographic recordings were initiated 1 day before tail suspension and continued daily throughout the suspension protocol. Data from the telemetric implants were monitored by placing a radio receiver under the study cage and then waiting 60 min to allow recovery before the recordings were begun. The receivers were fed via a data-exchange matrix into a personal computer using the Dataquest software analysis package (Data Sciences, St. Paul, MN). Electrocardiograph signals were recorded and digitized at a sampling rate of 1 kHz. Data were acquired for 30 min at the same time each afternoon to eliminate any effect of the circadian rhythm on heart rate. On the final day of suspension, a baseline recording was obtained, after which the mice were removed from the suspension apparatus and a second recording was obtained after 15 min.

Incremental Treadmill Exercise

Before suspension, baseline metabolic measurements during exercise were performed utilizing a Simplex II metabolic rodent treadmill (Columbus Instruments, Columbus, OH), which allows volumetric gas analysis. This treadmill is equipped with an Oxymax metabolic chamber for measurements of oxygen consumption (VO₂) and carbon dioxide production (VCO₂) using a closed-chamber volumetric method of gas analysis. The respiratory exchange ratio (RER) was calculated as the ratio of VO₂ to VCO₂. Mice were subjected to graded treadmill exercise per our laboratory’s previously described protocol (11). Briefly, mice were placed in the exercise chamber and allowed to equilibrate (usually 30–60 min). Treadmill activity was initiated at 3.5 m/min, 0° inclination. Treadmill speed and inclination were then increased by 2.5 m/min and 2° inclination every 3 min thereafter until mice stopped running from exhaustion. Exhaustion was defined when the mouse spent >50% of the time or >15 s consecutively on the shock grid.

Graded treadmill exercise was performed before both preimplantation of telemetric units and postimplantation to ensure that the mouse had returned to baseline level and to determine the influence of the telemetric unit (which weighs 3.5 g) on the exercise response. Exercise studies were also performed after 2 wk of tail suspension to determine the effects of simulated microgravity on exercise capacity and metabolic parameters.

Baroreceptor Studies

Anesthesia has been shown to markedly alter cardiovascular reflex activity in the mouse (11, 30). Thus, to avoid the influence of anesthesia on cardiovascular reflexes, all evaluations of baroreceptor reflex changes were performed in chronically instrumented, nonanesthetized mice. Because heart rate measurements were possible using the blood pressure tracing from the carotid artery catheter, these mice represented a separate subgroup that did not have telemetry implants to avoid the potential effects of multiple surgeries. After 2 wk of simulated microgravity, the mice were anesthetized with inhaled isoflurane (3%) induced in a closed chamber and maintained via nose cone (1–2%) throughout surgery. With the mouse supine, but maintained in a head-down position under the dissecting microscope (Nikon, Tokyo, Japan), a midline neck incision was made from just below the mandible to the thoracic inlet. The left carotid artery was identified, isolated from surrounding structures (jugular vein and vagus nerve), and secured with 4-0 silk suture. An Intramedic PE-10 polyethylene catheter (Clay Adams, Parsippany, NJ) was stretched to approximately one-half its original diameter by pulling between the thumb and finger. The catheter was advanced to an approximate depth of 1 cm and secured in place with 4-0 surgical silk suture (Ethicon) both proximal and distal to the vessel entry site. The catheter was then flushed with heparinized saline (100 U/ml saline), sealed with cyanoacrylate glue, tunneled to the back of the neck, and tucked into a subcutaneous pocket on the back. Skin incisions were sutured closed with 4-0 surgical silk. Ampicillin (100 mg/kg ip) was administered immediately after skin closure. Mice were then placed back in the suspension cage and allowed to recover for 24 h before the baroreflex studies, with food and water available ad libitum.

After removal from the subcutaneous pocket, the arterial catheter was connected to a Spectramed DTX Plus pressure transducer. Analog signals were amplified using a Gould (Cleveland, OH) model 11-1202-25 preamplifier and model 13-4615-52 amplifier. Analog signals were digitalized using a Data Translation (Marlboro, MA) Series DT2801 analog-to-digital converter and analyzed using Dataflow data-acquisition software (Crystal Biotech, Hopkinton, MA). Systolic and mean blood pressures were obtained from the blood pressure waveforms. Measurement of the heart rate was obtained using a software peak identification tachometer. Calibration of the transducer was performed immediately before each experiment with a standard mercury sphygmomanometer (Omron Healthcare, Vernon Hills, IL).

Mice were initially studied during suspension, in the quiet awake state, without anesthesia or sedation. After stable baseline heart rate and blood pressure measurements were recorded, baroreflex sensitivity was assessed by interarterial graded bolus doses (0.5, 0.75, 1.0, 2.5, 5.10, and 20 μg/kg) of sodium nitroprusside, a direct arterial vasodilator, to lower blood pressure. After recovery for 30 min and documented return to baseline hemodynamics, graded intra-arterial doses (1, 2, 5, 7.5 10, and 20 μg/kg) of phenylephrine, an arterial vasoconstrictor, were administered to raise blood pressure. Each injection was given only after heart rate and blood pressure had returned to baseline levels. Measurements of heart rate, R-R interval, and blood pressure were performed after each dose. This graded dose protocol was then repeated after the mouse was returned to the reloaded environment. Similar studies were performed on control mice that were instrumented identically but that were left unsuspended. Heart rate and blood pressure measurements were collected and averaged for each of the sodium nitroprusside and phenylephrine doses for the control, suspended, and unsuspended groups. Dose-response curves were generated using Prism Graphpad software (GraphPad Inc, San Diego, CA).

The slope of the R-R interval vs. mean arterial blood pressure, an index of baroreflex sensitivity, was determined by linear regression. In addition, the baroreflex operational point (resting R-R interval – minimum R-R interval)/R-R range × 100%, was calculated for control, suspended, and unsuspended mice. The operational point is a measure of the relative buffering capacity of the baroreflex above and below resting levels. If the operational point is low, there is relatively less buffering capacity during the hypertensive part of the range; if the operational point is high, there is relatively less buffering capacity during hypertension (15).

Statistical analysis was performed by Student’s t-test, using Bonferroni’s correction when comparing multiple time points, and by ANOVA with Fisher’s paired least significant difference post hoc testing when comparing multiple groups. Statistical significance was considered achieved at P < 0.05.
RESULTS

Chronotropic Response

The murine heart rate response to tail suspension is biphasic (Fig. 1). Mice (n = 15) demonstrate a moderate bradycardia, reaching a maximum 11% decrease from baseline after 2 days of simulated microgravity. This decrease in heart rate persists throughout the first 4 days (P < 0.01), after which heart rate returns to baseline level. After release from tail suspension (reloaded state), heart rate immediately increased by 15% over baseline (P < 0.01).

Blood Pressure Response

Mean arterial blood pressure increased slightly under tail suspension (n = 8) and dropped slightly after reloading, but none of these changes were statistically significant (Fig. 2). Blood pressures were also not significantly changed in the control (unsuspended) mice (n = 4).

Heart Weight and Body Weight

Mice for this experiment (n = 8) were tail suspended but did not undergo implantation of the electrocardiograph telemetry units so as not to influence weight gain. Body weight and heart weight were recorded after 2 wk of tail suspension. Controls (n = 6) were housed under the same cage conditions as the suspended mice. Absolute heart weights were not significantly altered after 2 wk of tail suspension (Fig. 3A), whereas absolute body weights were decreased (P < 0.02) (Fig. 3B). Thus heart weight normalized to body weight (heart weight-to-body weight ratio) demonstrated a 16% increase (P < 0.001) in the suspended mice compared with controls (Fig. 3C).

Exercise Capacity

Thirteen mice were subjected to the standard graded treadmill exercise protocol before tail suspension and then studied again immediately after release from 2 wk of suspension. Total exercise capacity, as measured by distance ran, decreased by 64% (P < 0.002; Fig. 4A). Peak VO2 and peak VCO2 were decreased by 7% (P < 0.07) and 11% (P < 0.01), respectively (Fig. 4, B and C). Although RER was minimally increased by 4%, this did not reach statistical significance.

Fig. 1. Murine heart rate response during 14 days of tail suspension-simulated microgravity and recovery. There is an initial mild bradycardia, with a return to normal resting heart rates by the end of the first week of suspension, followed by a pronounced tachycardia after reloading. Values are means ± SD. bpm, Beats/min. *P < 0.01 vs. baseline.

Fig. 2. Mean arterial blood pressure (BP) during presuspension, after 14 days of tail suspension, and after reloading. There were no significant changes in mean BP at any of these time points. Values are means ± SD; n, no. of animals.

Fig. 3. Body weight was decreased (A), heart weight was unchanged (B), and heart weight-to-body weight ratio was increased (C) after 14 days of tail suspension. Values are means ± SD; n, no. of animals. *P < 0.02. †P < 0.001.
Capacity for Vasoconstriction and Vasodilation

Phenylephrine stimulates peripheral arterial α-adrenergic receptors, resulting in a dose-dependent vasoconstriction and a reflex bradycardia secondary to an increase in vagal tone. Phenylephrine, at doses ranging from 1 to 20 μg/kg, was administered intra-arterially to measure the α-adrenergic responsiveness of the peripheral arterial bed during simulated microgravity and the subsequent return to the reloaded condition (n = 9). During tail suspension, there was a trend toward an attenuated response to phenylephrine at all doses compared with unsuspended controls (n = 4). After return to the reloaded condition, there was a trend toward an exaggerated response to phenylephrine compared with controls, although these differences did not reach statistical significance. This shift is depicted in the change in the sodium nitroprusside dose-response curve (Fig. 6).

Sodium nitroprusside, a nonendothelial smooth muscle arterial vasodilator, was also administered intra-arterially in a range of 0.5–20 μg/kg. During tail suspension (n = 9), the vasodilator response did not change compared with nonsuspended controls (n = 4); however, after return to the nonsuspended, reloaded condition, the vasodilatory response was significantly increased (P < 0.05; Fig. 7). This shift is depicted in the change in the sodium nitroprusside dose-response curve (Fig. 8).

Baroreceptor Sensitivity

The baroreflex response was abnormal during both tail suspension and after return to the reloaded state (Fig. 9). In tail-suspended mice, the operational point was 25.1 ± 14.6%, one-half of that in control mice (54.1 ± 1.8%; P < 0.007). The operational point decreased further after mice were returned to the reloaded state (17.2 ± 17.3%), 32% of the control value (P < 0.05).

DISCUSSION

Mice undergoing 2 wk of tail suspension-simulated microgravity manifest many of the cardiovascular alterations that have been previously demonstrated in humans during spaceflight (2, 4, 8, 14–18, 19) and under conditions of simulated microgravity and in rats during tail suspension (3, 10, 12, 27, 28, 35, 39, 42, 44). These include alterations in heart rate, capacity for vasodilation, and baroreceptor sensitivity. There are, however, some important differences between species.
Heart Rate and Blood Pressure Response

Early human studies during Spacelab missions demonstrated that in-flight heart rate was elevated only during the first 20–30 min then decreased during continued exposure to microgravity (2, 14). The length of exposure to microgravity conditions was also noted to alter the chronotropic response [e.g., during prolonged missions heart rates have been shown to increase (35)]. Postflight quiet standing heart rate is markedly elevated (4). Unlike in humans, rats subjected to simulated microgravity via tail suspension did not demonstrate an initial exaggerated heart rate response. In one study, it was observed that the heart rate increased slightly, although not significantly, on day 7 of tail suspension (27). In rats, heart rate response postsuspension has been variable, with some investigators finding an increase (21, 23, 38) and others not (3, 27). The murine model of simulated microgravity appears to simulate many of the heart rate responses that have been noted previously. Initially, mice show a slight decrease in heart rate, yet during further exposure to simulated gravity, heart rate increased to presupension levels. Similar to humans (and to many studies in rats), there was a dramatic increase in heart rate immediately after return to the reloaded condition.

There are much more subtle changes in supine blood pressure in humans during spaceflight. During microgravity conditions, diastolic pressure is decreased (18), but during the stress of reentry and landing, blood pressure increases. After return to the reloaded state, supine systolic blood pressure is slightly increased, but it returns to normal preflight levels after 24 h. In one study, supine diastolic blood pressure was unchanged postflight (17), whereas in another study it was found to be elevated (4). Rats subjected to tail suspension did not show a significant change in mean arterial blood pressure (3). Similarly, we have found that mice also did not show significant alterations in mean arterial blood pressure during or after simulated microgravity. Some of these differences between humans and rodents may reflect the differences between circulatory adjustments in bipeds and quadrupeds or the differences in large vs. small mammals. To the extent that fluid shifts from upper to lower body play a role in the adaptation to microgravity, the small body size of rodents may be a limiting factor for these adaptations.

Exercise Capacity

Exercise capacity is one of the best indicators of overall cardiovascular performance and reserve. During spaceflight, human maximal exercise capacity, as evaluated by peak VO$_2$ and peak power, is maintained for as long as 83 days. Postflight, however, exercise capacity markedly decreases, with peak VO$_2$ declining by 20% (14, 19). Rats show a similar decrease in exercise capacity after exposure to simulated microgravity: peak VO$_2$ is decreased by 14% and total distance run is decreased by 33% after 21 days of tail suspension (35, 39, 42). In mice, after 14 days of tail suspension, distance run decreased by an impressive 67%; however, the decrease in peak VO$_2$ was considerably less (7%).

Heart Weight and Heart Weight-to-Body Weight Ratio

Postflight cardiac dimensions have been estimated radiographically and by echocardiography in humans. Body weight decreases postflight, and heart size is decreased as estimated by
cardiothoracic ratio on chest radiographs, compared with pre-flight measurements (14). Echocardiographic studies have shown that left ventricular volume increases during the first 48 h of flight, then decreases, and remains decreased postflight (2, 14). In humans exposed to 14 days of head-down tilt, cardiac mass trended lower by 5% (but not significantly) (20). In rats, in which direct heart weight measurement is feasible, Ray et al. (28) have shown that heart mass is unchanged after 7 days of actual spaceflight or after 7 and 28 days of hindlimb unloading. Others have confirmed these results (39, 40), although some studies have shown diminished cardiac mass (42). Ray et al. have suggested that the cardiac atrophy found by some investigators does not result from hindlimb unloading but that it occurs when the loss of body mass is disproportionately large. In mice, body weight decreases by 28% after 14 days of tail suspension; however, heart weight does not change. Because of the decrease in total body mass, heart weight normalized to body weight (heart rate-to-body weight ratio) actually increases compared with controls.

Peripheral Vascular Reactivity

Altemations in vasoconstriction. In humans exposed to simulated microgravity, α-adrenergic receptor-mediated peripheral vasoconstriction has been shown to be enhanced (8). Afterward, however, there is an inability to increase peripheral vascular resistance (4, 26, 37), which may contribute to orthostatic intolerance. Rats under simulated microgravity demonstrate a decreased pressor response as well (9, 10, 23, 27). In contrast, in mice both during and after tail suspension, the pressor response to phenylephrine was not significantly altered.

Altemations in vasodilation. Humans during simulated microgravity show an increased vasodilatory response to administration of the β-adrenergic receptor agonist, isoproterenol (8). Shoemaker et al. (32) studied human subjects after 14 days of head-down tilt and determined that central modulation of sympathetic discharge and peripheral vascular adaptations occurred concurrently to maintain mean arterial pressure despite a decrease in sympathetic discharge. Arterioles isolated from rats during hindlimb suspension showed no changes in isoproterenol vasodilatory response, but diminished maximal responses to adenosine and sodium nitroprusside, suggesting that unloading-induced orthostatic hypotension and the associated inability to adequately elevate peripheral vascular resistance are not the result of an enhanced vasodilatory responsiveness of the skeletal muscle resistance vasculature (22). Woodman et al. (41) examined leg muscle arterioles obtained from rats undergoing hindlimb unloading and demonstrated decreased endothelium-dependent vasodilation, endothelial nitric oxide synthase expression, and superoxide dismutase-1 expression. However, after release from tail suspension, rat aortic rings show an increased vasodilator response to the nonspecific vasodilator sodium nitroprusside (10). In our study, mice during tail suspension did not show any alteration in vasodilatory response; however, after return to the reloaded condition, they showed an increased vasodilatory response, similar to that described in humans and rats. Our in vivo studies are partially limited by their reliance on whole organ responses. Future studies in vascular rings could provide additional information regarding vascular contractile response; however, in the mice, these studies can only be performed reliably in larger conductance vessels, thus representing only one segment of the total vasoreactive bed.

Baroreflex Sensitivity

There is evidence of decreased baroreflex buffering after exposure to microgravity; however, the importance of this in orthostatic intolerance is controversial. After a 4- to 5-day space shuttle mission, investigators found reductions in carotid baroreceptor-reflex responsiveness and correlated these with orthostatic intolerance (15). In astronauts who could not finish the postflight stand test, heart rate failed to continue to increase with progressive hypotension, suggesting a reduced range of baroreflex buffering. After longer missions (8–14 days), baroreflex slope, total range, and operational point were reduced postflight, and the degree of this reduction correlated with the degree of orthostatic intolerance (17). Buckey et al. (4) examined the etiology of orthostatic intolerance in astronauts performing a 10-min stand test before and after a 9–14-day space shuttle mission. They demonstrated that there was no deficit in the ability of the orthostatically intolerant astronauts to elevate heart rate and concluded that a lack of baroreflex responsiveness does not cause orthostatic intolerance.

In one study in rats undergoing tail suspension, baroreceptor sensitivity decreased slightly on day 7 and increased after return to the reloaded state (3). Our study demonstrates that mice undergoing simulated microgravity follow the baroreflex trend seen in humans. After 14 days of tail suspension, the operational point is significantly decreased and decreases even further after reloading. This decreased range of baroreflex buffering suggests that there is either a diminished capacity to increase heart rate by withdrawal of vagal tone or an alteration in sympathetic responsiveness. The mouse is, therefore, an excellent model for further studies on the mechanisms of this baroreflex abnormality.

Conclusion

In conclusion, the mouse demonstrates a wide range of cardiovascular responses to tail suspension-simulated microgravity, including alterations in heart rate, exercise capacity, peripheral arterial vasodilatory responsiveness, and baroreflex response. Many of these responses are qualitatively similar to those demonstrated in humans, both during spaceflight or simulated microgravity using head-down tilt and in rats using tail suspension. There are, however, important differences, notably the absence of impaired vasoconstriction in the mouse. The present study confirms the utility of the murine model for studies of cardiovascular changes during microgravity, with the caveat that species differences must always be considered. Whereas in the past the rat has been the principal small mammal utilized in these simulations, there are a number of reasons why the mouse may ultimately be a better model, including its much smaller size, resulting in reduction in payload weight and cost. Recent advances in microsurgical techniques, in microinstrumentation, and in microtelemetry make even highly complex physiological measurements feasible in the murine model. Finally, the ease of producing both transgenic and gene knockout models in the mouse, including now the ability to conditionally knockout a gene in a tissue-specific manner, gives researchers powerful new tools to study.
the mechanisms of cardiovascular adaptations to microgravity and to test the effectiveness of potential countermeasures.

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GRANTS

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