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Modulation of intracellular calcium transient in response to β-adrenoceptor stimulation in the hearts of 4-wk-old rats during simulated weightlessness

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Cui Y, Zhang S-M, Zhang Q-Y, Fan R, Li J, Guo H-T, Bi H, Wang Y-M, Hu Y-Z, Zheng Q-J, Gu C-H, Yu S-Q, Yi D-H, Li Z-C, Pei J-M. Modulation of intracellular calcium transient in response to β-adrenoceptor stimulation in the hearts of 4-wk-old rats during simulated weightlessness. J Appl Physiol 108: 838–844, 2010. First published February 4, 2010; doi:10.1152/japplphysiol.01055.2009.—Modulation of intracellular calcium ([Ca2+]i) transient in response to β-adrenoceptor stimulation in the hearts of hindlimb unweighted (HLU) rats during simulated weightlessness has not been reported. In the present study, we adopted the rat tail suspension for 4 wk to simulate weightlessness. Effects of simulated microgravity on β-adrenoceptor responsiveness were then studied. Mean arterial blood pressure, left ventricular pressure (LVP), systolic function [maximum positive change in pressure over time (+dP/dtmax)], and diastolic function [maximum negative change in pressure over time (−dP/dtmax)] were monitored during the in vivo experiment. β-Adrenoceptor density was quantitated by radioactive ligand binding. Single rat ventricular myocyte was obtained by enzymatic dissociation method. ±dP/dtmax, myocyte contraction, intracellular [Ca2+]i, transient, and L-type calcium current in response to β-adrenoceptor stimulation with isoproterenol were measured. Compared with the control group, no significant changes were found in heart weight, body weight, and mean arterial blood pressure, whereas LVP and ±dP/dtmax were significantly reduced. LVP and ±dP/dtmax were significantly attenuated in the HLU group in response to isoproterenol administration. In the in vitro study, the β-adrenoceptor density was unchanged. Effects of isoproterenol on electrically induced single-cell contraction and [Ca2+]i transient in myocytes of ventricles in HLU rats were significantly attenuated. The enhanced L-type Ca2+ current elicited by isoproterenol in cardiomyocytes was significantly decreased in the HLU group. The above results indicate that impaired function of L-type Ca2+ current and decreased [Ca2+]i transient cause the depressed responsiveness of the β-adrenoceptor stimulation, which may be partially responsible for the depression of cardiac function.

INCREASING EVIDENCE DEMONSTRATES that microgravity leads to reduced cardiac contractility (5, 19, 21). Depressed contractility may be due to the changes of cardiac tissue, low cardiovascular response to low circulating blood volume, and impaired regulation of cardiac function. It is well known that the β-adrenoceptor is a predominant receptor in regulation of cardiac function. Recent studies suggested that the responsiveness of cardiac contractility to β-adrenoceptor stimulation is reduced after weightlessness (10, 11). Depressed β-adrenoceptor responsiveness may be due to downregulation of the β-adrenoceptor itself or to the impaired postreceptor events. A previous study reported that density and affinity of β-adrenoceptor are not changed under simulated microgravity (4), but postreceptor events, such as Gs protein/adenylyl cyclase (AC)/cAMP/protein kinase A/Ca2+ cascades, which may also be responsible for the depressed β-adrenoceptor responsiveness, are still not well understood.

In our laboratory’s previous study, we showed that the function of the Gs protein, which mediates the action of β-adrenoceptor stimulation (7, 9, 11), is not altered during weightlessness (17). The function of AC, the enzyme activated by the Gs protein and, in turn, converts ATP into cAMP, is impaired during weightlessness. We have also documented that weightlessness is associated with reductions in cellular cAMP and impaired AC response to forskolin (17), an activator of AC (12). When facilitated by β-adrenoceptor stimulation during excitation and contraction coupling, whether or not downstream Ca2+ cascade during weightlessness is changed warrants study.

Both in vivo and in vitro, in the present study, we delineated the postreceptor signaling mechanisms in the hearts of rats subjected to tail suspension for 4 wk, which has previously been shown to reduce cardiac contractility (21). With the use of maximum positive and negative change in pressure over time (±dP/dtmax) in vivo and electrically stimulated twitch amplitude and intracellular Ca2+ concentration ([Ca2+]i) transient in isolated ventricular myocytes in vitro as parameters, we determined the changes in ventricular myocytes of hindlimb unweighted (HLU) and control rats subjected to manipulations that activated β-adrenoceptor with isoproterenol. We also measured L-type Ca2+ current (ICa) in the hearts of control and HLU rats. Results from this study have provided evidence for the first time that the L-type ICa and [Ca2+]i transient in response to isoproterenol are decreased after HLU, which may be responsible for depressed β-adrenoceptor responsiveness during the simulated weightlessness.

MATERIALS AND METHODS

Animal care and animal models. Tail-suspended, HLU rat model (3, 19, 21) was used to simulate microgravity in this study. Male Sprague-Dawley (SD) rats weighing 150–180 g were obtained from the animal center of the Fourth Military Medical University, Xi’an, China, at the start of the experiment and were randomly divided into two groups. One group of rats (n = 44) was subjected to tail suspension to simulate microgravity, whereas the control (normal group, n = 44) was maintained at ambient air. The technique of tail...
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suspension (3) with modifications from our collaborative laboratory has been previously described in detail (10, 13). Animals were maintained at about −30°C head-down tilt, with their hindlimbs unloaded. All animals received standard laboratory chow and water ad libitum and were caged individually in a room maintained at 23°C with a 12:12-h light-dark cycle. The HLU period was 4 wk. All aspects of this study were reviewed and approved by the Animal Care and Use Board of the Fourth Military Medical University.

Surgical procedure for in vivo experiments. After SD rats were anesthetized with pentobarbital sodium (45 mg/kg ip), the surgical procedure was performed as previously described (12). The trachea was intubated and connected to a rodent ventilator (Jiangwan I Ventilator; Second Military Medical University) for artificial ventilation with room air (stroke volume, 10 ml/kg; 60 strokes/min). The temperature of the heating pad was adjusted to 37°C by a temperature controller. Arterial blood pressure was continuously monitored via a saline-filled catheter (PE-50; Becton Dickinson, Franklin Lakes, NJ) inserted into the right femoral artery, which was connected to a pressure transducer (AB-621G, Nihon Kohden, Tokyo, Japan). ECG and heart rate were measured by standard limb lead II electrodes using an isolated ECG bioamplifier (V75-04; Coulbourn Instruments, Allentown, PA). PE-50 catheters (Becton Dickinson) were inserted into the right external jugular vein of each rat for drug administration and into the left ventricle (LV) from the left carotid artery for measurement of LV systolic pressure (LVP), systolic function (+dP/dt max), and diastolic function (−dP/dt max) with a pressure transducer (AB-621G, Nihon Kohden). All signals were sent to a recording system (RM6200, Nihon Kohden). Following stabilization for 15 min, the animal was injected with vehicle or isoproterenol (15 μg/kg iv) through the left external jugular vein. The following determinations were carried out by other investigators blinded to the groups.

β-Adrenoceptor quantification. Rats were anesthetized by intraperitoneal injection (45 mg/kg) of pentobarbital sodium. The hearts were excised and rinsed with an ice-cold saline solution; the atria and ventricles were dissected, blotted dry, and weighed. LVs were immediately frozen in liquid nitrogen and stored at −80°C.

Fraction of the LV homogenates (100 μg) were incubated at room temperature for 90 min in a final volume of 500 μl in the presence of [125I]cyanopindolol as a radioligand (200 pM, specific activity: 2,445 Ci/mM) in fresh Joklik solution with 1% BSA. More than 70% of the cells sedimented by centrifugation at 100 g for 1 min, and resuspended in fresh Joklik solution with 1% BSA. Values are means ± SD in kPa; n = 12 rats/group. MABP, mean arterial blood pressure; LVP, left ventricular pressure; ISO, isoproterenol. *P < 0.01 compared with normal group. †P < 0.05 and ‡P < 0.01 compared with corresponding control group.

Table 1. Body and heart weights in normal and HLU rats

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<th>Normal</th>
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<tr>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>302.8 ± 12.4</td>
<td>286.9 ± 13.6</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>932.1 ± 32.7</td>
<td>928.5 ± 26.2</td>
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Values are means ± SD; n, no. of normal and hindlimb unweighted (HLU) rats.

Fig. 1. Inhibitory effects of hindlimb unweighting (HLU) in isoproterenol-induced elevation in left ventricular (LV) positive and negative maximum change in pressure over time (+LV dp/dt max). Group results show the effect of 15 μg/kg iv isoproterenol administration on +LV dp/dt max (A) and −LV dp/dt max (B) in normal and HLU rats with the control (Con) group as 100%. Values are means ± SD; n = 12 in each group. **P < 0.01 vs. corresponding Con; ###P < 0.01 vs. normal rats.
with 1% dialyzed BSA and a gas phase of 95% O2-5% CO2 (pH 7.4). The myocyte was field electrically stimulated at a rate of 0.2 Hz with platinum electrodes connected to a voltage stimulator. Twitch amplitude was measured with an automatic video analyzer (18). As a prerequisite for the proper operation of this automatic analyzer, cardiac myocyte image was projected on the video camera (SSCM370CE; Sony) and observed on the video monitor (PVM-145E; Sony) and was first rotated to align horizontally and parallel to each of the video raster lines. This was achieved by interposing a k-mirror (or a dove prism) between the microscope eyepiece and the video camera. The video analyzer was interposed between the video camera and the video monitor, and it generated a positionable rectangular window that was observed on the video monitor, together with the image of the cell. Light-dark contrast at the edge of the myocyte provided a marker for measurement of the amplitude of motion. The amplitude of the marker was directly proportional to the dark image of the contraction, and the action was performed in real time. Traces of twitch amplitude were recorded with the use of a two-channel amplifier recording system. The amplitude of myocyte motion remained unchanged for at least 10 min, indicating the stability of the preparation.

Measurement of intracellular Ca2+ and L-type I(Ca). To explore Ca2+ homeostasis in response to β-adrenoceptor stimulation, intracellular Ca2+ transient and I(Ca) actions were investigated in ventricular myocytes. [Ca2+]i was measured using fura-2 as the Ca2+ indicator. Ventricular myocytes were loaded with the cell-permeant indicators (5 μM) at room temperature (20–22°C) for 30 min and then superfused at 37°C with Tyrode solution. Cells were illuminated at 340 and 380 nm, and fluorescent light was collected over a range of 400–510 nm. [Ca2+]i transients were measured when cells were field stimulated at 0.5 Hz. Fura-2 signals were not calibrated, and results are expressed as a ratio of fluorescent light on excitation at 340 and 380 nm (340 nm-to-380 nm ratio).

L-type I(Ca) was measured with patch electrodes (2–4 MΩ) in whole-cell mode (Axopatch IC, Axon Instruments, Molecular Devices, Sunnyvale, CA) containing the following: CsCl (130 mM), glucose (11.5 mM), HEPES (6.0 mM), tetraethylammonium-chloride (5.0 mM), EGTA (1.0 mM), MgCl2 (2.9 mM), and ATP (3.0 mM) (pH 7.1). I(Ca) was elicited after a preconditioning pulse from −80 to −40 mV using 150-ms steps in 8-mV increments up to +40 mV. I(Ca) magnitude was the difference between peak inward current and that before the clamp step. No leak subtraction was used, and currents were blocked by 5 μM nicardipine or 5 mM NiCl2. I(Ca) magnitude as a function of potential was used to determine the voltage-dependent activation.

Statistical analysis. Values are expressed as means ± SD. In experiments concerning determination of twitch amplitude, one to four myocytes from a single rat were used. Values obtained from more than one myocyte were averaged, and the mean was used as a single entity for statistical analysis. Unpaired Student’s t-test or ANOVA

<table>
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<tr>
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<th>HLU</th>
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<td>Normal</td>
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<td>Isoproterenol (1 μM)</td>
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<td>Isoproterenol (1 μM)</td>
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Fig. 2. Effects of isoproterenol on the twitch amplitude of contraction in single ventricular myocytes of normal and HLU rats. A: representative tracings showing the recording of electrically induced contraction of myocytes in normal rats for 30 min (top) and the effect of 1 μM isoproterenol in single myocytes in normal (middle) and HLU (bottom) rats. B: group results showing the dose-related effects of isoproterenol in myocytes of normal and HLU rats with the Con group as 100%. For measurement of cell contraction, the ventricular myocyte was superfused with Krebs solution, and the myocyte was then electrically stimulated before administration of isoproterenol. The electrically induced contraction was recorded for 20 min after administration of isoproterenol. Twenty myocytes from five rats were used in each group. Values are means ± SD; n = 5. The baseline value of twitch amplitude of myocytes of HLU rats was 8.23 ± 0.14 μm, which is significantly lower at P < 0.01 compared with the corresponding value in normal rats (10.48 ± 0.16 μm). *P < 0.01 vs. Con. #P < 0.01 vs. corresponding Con with isoproterenol in normal rats.

Table 3. Protein concentration and β-adrenergic receptor densities

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<tr>
<td>n</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Total protein, mg/g</td>
<td>61.8 ± 4</td>
<td>58.6 ± 3</td>
</tr>
<tr>
<td>β-adrenergic receptor density, fmol/mg</td>
<td>56.4 ± 7</td>
<td>54.2 ± 5*</td>
</tr>
<tr>
<td>Kd, pM</td>
<td>34 ± 7.1</td>
<td>28 ± 5.6*</td>
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Values are means ± SD; n, no. of rats. Total protein concentration (mg/g of tissue) was determined from normal control and hindlimb left ventricular homogenates, as described in MATERIALS AND METHODS. Receptor number was expressed as fmol/mg protein. The affinity (Kd in pM) of radioligand [3H]quinuclidinyl benzilate was determined from Scatchard analyses. *Not significant.
was used to determine the differences among groups; \( P < 0.05 \) was considered statistically significant.

**RESULTS**

Body and heart weights in normal and HLU rats. Body and heart weights are shown in Table 1. After 4-wk tail suspension, body and heart weights were not significantly changed.

**Effects of 4-wk HLU on hemodynamics in anesthetized rats.** Hemodynamic parameters were continuously recorded. Table 2 and Fig. 1 summarize mean arterial blood pressure (MABP), LVP, systolic function \((+\mathrm{dP}/\mathrm{d}t_{\text{max}})\), and diastolic function \((-\mathrm{dP}/\mathrm{d}t_{\text{max}})\) in the two groups. MABP was unchanged, whereas LVP and \(\pm\text{LVdP}/\text{d}t_{\text{max}}\) were significantly decreased after 4-wk tail suspension \((P < 0.01)\).

**Effects of 4-wk HLU on the cardiac inotropic response to isoproterenol in anesthetized rats.** In both normal and HLU animals, \(\pm\text{LVdP}/\text{d}t_{\text{max}}\) was significantly increased following a 15-\(\mu\text{g/kg}\) iv dose of isoproterenol (adopted according to Ref. 8) \((P < 0.01)\). However, these maximum inotropic responses were significantly attenuated in the HLU group (Table 2, Fig. 1; \(P < 0.01\)). In addition, the response of MABP following isoproterenol administration was blunted in the HLU animals and was significantly decreased in the control animals.

**Effects of isoproterenol on the electrically induced contraction in single ventricular myocytes of normal and HLU rats.** Electrical stimulation triggered myocyte contraction, and the contractile responses were significantly lower in the HLU animals than in the control rats (Fig. 2A), consistent with the well-established theory that contractility is reduced after in vivo suspension. Isoproterenol, a \(\beta\)-adrenoceptor agonist, at a range of 0.01–10 \(\mu\)M, dose dependently increased the electrically induced contraction in the isolated single ventricular myocyte (Fig. 2). This response was blocked by 1 \(\mu\)M propranolol, a \(\beta\)-adrenoceptor antagonist (data not shown). In ventricular myocytes isolated from the ventricle of HLU rats, effects of isoproterenol on the contraction of the myocyte were significantly attenuated, indicating that the \(\beta\)-adrenoceptor desensitization occurred in the heart of HLU rats.

**\(\beta\)-adrenoceptor densities.** Figure 3 shows a typical Scatchard representation and illustrates the unmodified density of

![Graphs and figures](http://jap.physiology.org/)

**Fig. 4.** Effects of isoproterenol (Iso) on the electrically induced intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) transient in single ventricular myocytes of normal (Nor) and HLU rats. A: representative tracings showing the recording of electrically induced [Ca\(^{2+}\)]\(_i\) transient of myocytes in normal rats. B: effects of 1 \(\mu\)M isoproterenol in normal rats. C: representative tracings showing the recording of electrically induced [Ca\(^{2+}\)]\(_i\) transient of myocytes in HLU rats. D: effects of 1 \(\mu\)M isoproterenol in normal rats. E: group results showing the dose-related effects of isoproterenol in myocytes of normal and HLU rats with the Con group as 100%. For measurement of electrically induced [Ca\(^{2+}\)]\(_i\) transient, the ventricular myocyte was superfused with Krebs solution, and the myocyte was then electrically stimulated before administration of isoproterenol. The electrically induced [Ca\(^{2+}\)]\(_i\) transient was recorded for 20 min after administration of isoproterenol. Twenty myocytes from five rats were used in each group. Values are means \(\pm\) SD; \(n = 5\). The baseline value of amplitude of [Ca\(^{2+}\)]\(_i\) transient in myocytes of HLU rats was 0.38 \(\pm\) 0.09 (fluorescence ratio), which is significantly lower at \(P < 0.01\) compared with the corresponding value in normal rats (0.68 \(\pm\) 0.21). **\(P < 0.01\) vs. Con. ***\(P < 0.01\) vs. corresponding Con with isoproterenol in normal rats.
β-adrenoceptor in LVs of the HLU rat compared with control. Results are shown in Table 3. Both the mean density and $K_d$ of β-adrenoceptor were unchanged in LVs of HLU rat compared with controls (Table 3).

Effects of isoproterenol on the electrically induced \([Ca^{2+}]_i\) transient in single ventricular myocytes of normal and HLU rats. Experiments on the contractile response in the present study suggested that β-adrenoceptor signaling cascades, namely, $G_i$ protein/AC/cAMP/PKA/Ca$^{2+}$, may be impaired in the heart of HLU rats. To further determine whether Ca$^{2+}$ homeostasis was impaired in the heart of HLU rats, we measured the electrically induced \([Ca^{2+}]_i\) transient in the myocytes of HLU rats. The \([Ca^{2+}]_i\) transient was significantly decreased compared with the normal group (Fig. 4, A and C). It was also shown that isoproterenol, at the range of 0.001–1 μM, dose dependently increased the electrically induced \([Ca^{2+}]_i\) transient in the isolated, single ventricular myocytes (Fig. 4, B, D, and E). In ventricular myocytes isolated from the ventricles of HLU rats, effects of isoproterenol on the \([Ca^{2+}]_i\) transient of the myocyte were significantly attenuated, indicating that Ca$^{2+}$ signaling after β-adrenoceptor stimulation may be impaired in the heart of HLU rats.

Effects of isoproterenol on the L-type \(ICa\) in single ventricular myocytes of normal and HLU rats. To further determine whether decreased Ca$^{2+}$ response to β-adrenoceptor stimulation is related to the L-type \(ICa\), we measured the L-type \(ICa\) in the myocytes of normal and HLU rats (Figs. 5, A–C, and 6). It was also shown that isoproterenol, at the range of 0.001–1 μM, dose dependently increased the L-type \(ICa\) in the isolated single ventricular myocytes in both groups (Fig. 6). However, in ventricular myocytes isolated from the ventricle of HLU rats, effects of isoproterenol on the L-type \(ICa\) were significantly attenuated, indicating that L-type \(ICa\) in response to β-adrenoceptor stimulation may be impaired in the heart of HLU rats.

**DISCUSSION**

The most interesting observations in the present study are as follows: 1) β-adrenoceptor density is not changed, and cardiac function in response to isoproterenol, which stimulates the β-adrenoceptor on LVP and $±LVdP/dt_{max}$, is significantly attenuated in HLU rats; and 2) the stimulatory actions of isoproterenol on the electrically induced contraction and \([Ca^{2+}]_i\) transient and on L-type \(ICa\) in the ventricular myocytes are significantly attenuated in HLU rats. These observations indicate that the reduced responsiveness to stimulation in HLU rats is due, at least partially, to an impaired Ca$^{2+}$ homeostasis and impaired function of L-type Ca$^{2+}$ channel.

Depressed β-adrenoceptor responsiveness may be due to downregulation of the β-adrenoceptor itself, or to the impaired postreceptor events. A previous study has reported that density and affinity of β-adrenoceptor are not changed under the simulated microgravity (4). Together with the present study results that the β-adrenoceptor remains unchanged, the reduced responsiveness to β-adrenoceptor stimulation is probably due to impaired postreceptor signaling mechanisms.

In the present study, we delineated the postreceptor signaling mechanisms in the hearts of rats subjected to tail suspension for 4 wk, which has previously been shown to reduce cardiac contractility (19). With the use of LVP and $±dP/dt_{max}$ in vivo and electrically stimulated twitch amplitude, \([Ca^{2+}]_i\) transient, and L-type \(ICa\) in isolated ventricular myocytes in vitro as parameters, we determined the changes in HLU and control rats subjected to manipulations that activated β-adrenoceptor with isoproterenol. The electric-
cally induced [Ca\(^{2+}\)], transient represents the influx of Ca\(^{2+}\) via the L-type Ca\(^{2+}\) channel upon electrical stimulation, which then triggers release of Ca\(^{2+}\) from the sarcoplasmic reticulum, leading to a [Ca\(^{2+}\)] transient and muscle contraction. A previous study in our laboratory showed that, under similar experimental conditions, the electrically induced [Ca\(^{2+}\)], transient is directly related to contractility (15).

In our in vivo study, MABP was unchanged, whereas LVP and ±LVdP/dt\(_{max}\) were all significantly decreased after 4-wk HLU. Responses of MABP, LVP, and ±LVdP/dt\(_{max}\) to isoproterenol were significantly attenuated in HLU animals. In agreement with the recent studies in 2-wk HLU mice (10) and 4-wk HLU rats (20), experiments on the myocyte contractile responses to isoproterenol in vitro in the present study demonstrated that contractile responses to isoproterenol were impaired in myocytes isolated from HLU rats. The results suggested that some downstream sites in β-adrenoceptor signaling cascades, namely, G\(_s\) protein/AC/cAMP/PKA/Ca\(^{2+}\)/contraction, may be impaired in the heart of HLU rats.

In our laboratory’s previous study (17), we determined G\(_s\) protein and the function of AC under simulated microgravity. It was shown that the biologically active isoform, G\(_{\text{max}-\text{small}}\), was not changed after 4 wk of HLU. Our previous study provided evidence for the first time that, after 4-wk HLU, which induces simulated weightlessness, the attenuated cardiac response to β-adrenoceptor stimulation is due to impaired function of AC. We have also postulated that, for downstream AC homeostasis and impaired function of L-type Ca\(^{2+}\) channel.

In addition, an early explanation for the depressed contractility after simulated weightlessness is that it may be due to cardiac tissue atrophy (19), which is induced by 90-day (long-term) HLU. It is noteworthy that, in our present study, heart and body weights were not significantly changed after 4 wk of HLU. It is possible that the depressed contractility may not be due to atrophy, which did not exist after 4-wk HLU.

It should be stated that the results of our present study in 4-wk HLU rats are different from the results in head-down bed rest in humans, described in previous studies (1, 6). Data in these studies provided evidence that simulated microgravity for 5 or 14 days causes an increase in β-adrenoceptor responsiveness. The phenomenon of different results from different species is very interesting, and time course study of the microgravity in HLU rats (especially the microgravity for 5 or 14 days) is warranted.

Conclusions. The present study has provided first-time evidence that, after 4-wk HLU, which induces simulated weightlessness, the attenuated cardiac response to β-adrenoceptor stimulation is at least partially due to an impaired Ca\(^{2+}\) homeostasis and impaired function of L-type Ca\(^{2+}\) channel in rats.

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DISCLOSURES

No conflicts of interest are declared by the author(s).
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