Differential regulation of L-type Ca\textsuperscript{2+} channels in cerebral and mesenteric arteries after simulated microgravity in rats and its intervention by standing

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Xue J-H, Zhang L-F, Ma J, Xie M-J. Differential regulation of L-type Ca\textsuperscript{2+} channels in cerebral and mesenteric arteries after simulated microgravity in rats and its intervention by standing. Am J Physiol Heart Circ Physiol 293: H691–H701, 2007. First published March 9, 2007; doi:10.1152/ajpheart.01229.2006.—This study was designed to clarify whether simulated microgravity can induce differential changes in the current and protein expression of the L-type Ca\textsuperscript{2+} channel (Ca\textsubscript{L}) in cerebral and mesenteric arteries and whether these changes can be prevented by daily short-duration –G\textsubscript{y} exposure. Tail suspension [hindlimb unloading (HU)] for 3 and 28 days was used to simulate short- and medium-term microgravity-induced deconditioning effects. Standing (STD) for 1 h/day was used to provide –G\textsubscript{y} as a countermeasure. Whole cell patch-clamp experiments revealed an increase in current density of Ca\textsubscript{L} of vascular smooth muscle cells (VSMCs) isolated from cerebral arteries of rats subjected to HU and a decrease in VSMCs from mesenteric arteries. Western blot analysis revealed a significant increase and decrease of Ca\textsubscript{L} channel protein expression in cerebral and small mesenteric arterial VSMCs, respectively, only after 28 days of HU. STD for 1 h/day did not prevent the increase of Ca\textsubscript{L} current density in cerebral arterial VSMCs, but it prevented completely (within 3 days) and partially (28 days) the decrease of Ca\textsubscript{L} current density in small mesenteric arterial VSMCs. Consistent with the changes in Ca\textsubscript{L} current, STD for 1 h/day did not prevent the increase of Ca\textsubscript{L} expression in cerebrovascular myocytes but did prevent the reduction of Ca\textsubscript{L} expression in mesenteric arterial VSMCs subjected to 28 days of HU. These data indicated that simulated microgravity up- and downregulates the current and expression of Ca\textsubscript{L} in cerebral and hindquarter VSMCs respectively. STD for 1 h/day differentially counteracted the changes of Ca\textsubscript{L} function and expression in cerebral and hindlimb arterial VSMCs of HU rats, suggesting the complexity of the underlying mechanisms in the effectiveness of intermittent artificial gravity for prevention of postflight cardiovascular deconditioning, which needs further clarification.

postflight cardiovascular deconditioning; hindlimb unloading; vascular smooth muscle cells; calcium channels; countermeasure; intermittent artificial gravity

DURING MICROGRAVITY EXPOSURE, all gravitational blood pressure gradients disappear; therefore, blood vessels in dependent body regions are chronically exposed to lower-than-normal upright 1-G blood pressures, whereas those in the upper body regions are exposed to higher-than-normal upright 1-G blood pressures (15, 39, 45). Studies in rats have revealed that simulated microgravity differentially alters the function and structure of cerebral and hindquarter vessels. Attenuated myogenic tone, attenuated vasoreactivity, and atrophy were found in hindquarter arteries, whereas an enhanced myogenic tone and vasoreactivity and hypertrophy have been demonstrated in cerebral vessels (7, 12, 23–25, 43, 45, 49). These findings support the hypothesis that microgravity-induced redistribution of transmural pressures across the arterial vasculature can induce region-specific vascular adaptation (15, 39, 45). Additionally, recent studies in humans subjected to bed rest or spaceflight have found that the inability to adequately elevate the peripheral resistance (1, 2) and the altered autoregulation of cerebral vasculature (11) are important factors in postflight orthostatic intolerance (45). Our previous work suggests that different profiles of channel remodeling involving K\textsuperscript{+} and Ca\textsuperscript{2+} channels in arterial vascular smooth muscle cells (VSMCs) may play a role in mediating differential vascular adaptation during microgravity (9, 44). However, in our previous work (44), we investigated the changes of L-type Ca\textsuperscript{2+} channels (Ca\textsubscript{L}) by the whole cell patch-clamp technique only in cerebrovascular myocytes from rats after 28 days of simulated microgravity. Thus it is important to extend the observation by including VSMCs from hindquarter vessels and to determine protein expression and current density of Ca\textsubscript{L} concurrently (30, 34). Furthermore, it is important to elucidate whether changes in Ca\textsubscript{L} function and expression are among the early responses to microgravity (41).

Intermittent artificial gravity (IAG) induced by incorporation of a short-arm centrifuge into the spacecraft has been proposed as a promising multisystem countermeasure in future long-duration exploration-class spaceflight. We have shown that daily short-term (1 h) standing (STD), which mimics the physiological effect of IAG, is sufficient to prevent the differential adaptive changes in function and structure of vessels in different anatomic regions (35) and the postsusension cardiovascular dysregulation induced by a medium-term simulated microgravity in conscious rats (4). These findings are also consistent with several ground-based human studies suggesting potential benefits of IAG in prevention of cardiovascular deconditioning due to microgravity exposure (32, 36–38). Therefore, it is of interest to further investigate the alterations in vascular Ca\textsubscript{L} currents and protein subunit expression in rats subjected to this STD intervention during simulated microgravity. This could also provide further insight into the mechanism of vascular remodeling.

The present study was designed to investigate the changes in Ca\textsubscript{L} currents and protein expression of VSMCs isolated from cerebral and mesenteric arteries of rats exposed to 3 and 28 days of simulated microgravity compared with those of respective control rats. In addition, we investigated whether the differential changes in Ca\textsubscript{L} currents and protein expression due to simulated microgravity would be affected by the intervention of STD for 1 h/day.

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Materials and Methods

Animal Model and Experimental Design

Tail-suspended, hindlimb-unloaded rat model. The technique of tail suspension (26) with modification from our laboratory has been described in detail previously (3, 49). The animals were maintained at about −30° 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 on a 12:12-h light-dark cycle.

Model of daily short-duration −Gₙ exposure. Daily stationary ground support, or STD, for 1 h was adopted to simulate the effect of IAG, as previously described (35, 48). For short-duration STD exposure, the suspended rat was released from suspension and then placed in a 50-cm-long, tubelike metallic mesh cage maintained in a horizontal position for 1 h. The rat could move forward and backward but could not turn around. Food and water were provided ad libitum at the front end of the cage. The gravity vector was in the −Gₙ (dorsal-to-ventral) direction.

Experimental design. All protocols and procedures were reviewed and approved by the Animal Care and Use Committee of the Fourth Military Medical University. Two separate protocols were carried out.

In the first series of experiments (protocol 1), changes in C₄ₙ currents of VSMCs isolated from cerebral and mesenteric arteries of rats subjected to simulated microgravity with and without the STD countermeasure were examined and compared with those of respective control rats. Protocol 1 incorporated two sets of experiments. In experiment 1, changes in C₄ₙ currents and the effect of intervention were determined over 3 days of simulated microgravity. Thirty-six male Sprague-Dawley rats were randomly assigned to three experimental groups (n = 12 rats/group): control (Con), tail suspension (Sus), and suspension and for 1 h/day (Sus + STD). In experiment 2, differential changes in C₄ₙ currents of cerebral and mesenteric arterial VSMCs due to hindlimb unloading and the counteracting effect of STD for 1 h/day were evaluated over 28 days of simulated microgravity. Thirty-six male Sprague-Dawley rats were randomly assigned to three experimental groups (n = 12 rats/group): Con, Sus, and Sus + STD.

A second series of experiments (protocol 2) was designed to examine C₄ₙ protein expression. It incorporated two sets of experiments examining C₄ₙ and −Gₙ-subunit expression in cerebral and mesenteric arteries in response to simulated microgravity and effects of the STD countermeasure over 3 and 28 days. In each experiment, 48 male Sprague-Dawley rats were randomly assigned to three groups (n = 16 rats/group): Con, Sus, and Sus + STD.

The animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and killed by exsanguination via the abdominal aorta, and the left soleus and tibia were prepared as a stock solution in 100% ethanol and diluted to 0.1 mg/ml in 1% Triton X-100. For Western Blotting, membrane protein samples of cerebral and mesenteric arteries were prepared according to published methods (20, 22). Brieﬂy, isolated arterial specimens were minced into small pieces and homogenized on ice in a glass tissue grinder containing tissue protein extraction reagent (T-PER, Pierce) and protease inhibitor (Halt, Pierce). Arteries were generally pooled from four rats to provide higher protein yields. Large tissue debris and nuclear fragments were removed by two 100,000-g centrifugations and the samples were resuspended in sample buffer. Tissue samples were heated at 100°C for 10 min and loaded on SDS-PAGE. Proteins were transferred to nitrocellulose membranes and the membranes were probed with antibodies against C₄ₙ and −Gₙ-subunit.

Patch-Clamp Recording of C₄ₙ Currents

Cell preparation. The superior, middle, and basilar cerebral arteries with the circle of Willis and the superior mesenteric arteries with its branches were removed and placed in cold (4°C) physiological saline solution (PSS). The left soleus and tibia were removed, and the muscle wet weight and bone length were measured to confirm the efficacy of deconditioning and to monitor any effects on growth.

Values of body weights are means ± SD; others are means ± SE (n = 12). Con, simultaneous control; Sus, tail suspension; STD, standing; Sus + STD, 23 h/day Sus + 1 h/day STD. *P < 0.05; †P < 0.01 vs. Con.

Table 1. Body weight, soleus wet weight, and femur length in Con, Sus, and Sus + STD rats: protocol 1

<table>
<thead>
<tr>
<th>Body Wt. g</th>
<th>Initial</th>
<th>Final</th>
<th>Left Femur Length, mm</th>
<th>Left Soleus Wet Wt, mg</th>
</tr>
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<tbody>
<tr>
<td>Con</td>
<td>238.1 ± 7.6</td>
<td>261.7 ± 6.1</td>
<td>35.2 ± 0.3</td>
<td>112.8 ± 5.2</td>
</tr>
<tr>
<td>Sus</td>
<td>240.5 ± 8.8</td>
<td>256.9 ± 9.2</td>
<td>35.1 ± 0.2</td>
<td>89.6 ± 4.8†</td>
</tr>
<tr>
<td>Sus + STD</td>
<td>238.8 ± 6.7</td>
<td>253.6 ± 10.2</td>
<td>34.9 ± 0.3</td>
<td>110.1 ± 3.9</td>
</tr>
<tr>
<td>Con</td>
<td>237.9 ± 9.5</td>
<td>368.8 ± 8.7</td>
<td>36.2 ± 0.07</td>
<td>157.5 ± 2.8</td>
</tr>
<tr>
<td>Sus</td>
<td>240.3 ± 8.8</td>
<td>360.3 ± 9.5</td>
<td>36.0 ± 0.5</td>
<td>68.9 ± 2.1†</td>
</tr>
<tr>
<td>Sus + STD</td>
<td>242.3 ± 10.3</td>
<td>345.2 ± 9.6†</td>
<td>35.9 ± 0.9</td>
<td>99.0 ± 2.4†</td>
</tr>
</tbody>
</table>

Membrane protein samples of cerebral and mesenteric arteries were prepared according to published methods (20, 22). Brieﬂy, isolated arterial specimens were minced into small pieces and homogenized on ice in a glass tissue grinder containing tissue protein extraction reagent (T-PER, Pierce) and protease inhibitor (Halt, Pierce). Arteries were generally pooled from four rats to provide higher protein yields. Large tissue debris and nuclear fragments were removed by two
were incubated for 3 h with a 1:200 dilution of polyclonal rabbit anti-α1,c-(848–865) (Alomone Laboratories, Jerusalem, Israel), which is a sequence-directed antibody raised against amino acids 848–865 of the pore-forming α1,c-subunit of the CaL channel. The polyclonal antibody was diluted using PBS containing 0.1% Tween 20 and 5% nonfat dry milk. The membranes were then incubated for 45 min with infrared-labeled secondary antibodies (LI-COR) in PBS containing 0.1% Tween 20 and 0.01% SDS. A monoclonal mouse antibody raised against the structural protein β-actin (Sigma) was used as a lane-loading control (22). The bound antibody was detected by the Odyssey infrared imaging system (LI-COR), and the densities of the doublet bands at 200 and 240 kDa were summed to evaluate the level of α1,c-subunit expression. The densities of immunoreactive bands associated with anti-α1,c-(848–865) were expressed as percentage of the β-actin density for each lane.

Statistical Analysis

Values are means ± SE (except for body weight data, which are means ± SD). One-way ANOVA was used to determine the overall differences, and then Student-Newman-Keuls post hoc test was used to determine group differences. The 0.05 level of probability was chosen as significant for all analyses.

RESULTS

Body Weight, Soleus Wet Weight, and Femur Length

Body weight, soleus wet weight, and femur length data are summarized in Tables 1 and 2. Except for 6% less final body weight \((P < 0.05)\) in Sus + STD1 for 28 days than in Con rats, there were no significant differences between groups in each experiment. After 3 and 28 days of simulated microgravity, the soleus wet weight was 20% and 52% less, respectively, than in

<table>
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<tr>
<th>Experiment 1 (3 days simulated microgravity)</th>
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<tbody>
<tr>
<td>Con 225.3±7.2</td>
<td></td>
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</tr>
<tr>
<td>Sus 231.2±10.8</td>
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<tr>
<td>Sus + STD1 228.9±9.6</td>
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<th>Experiment 2 (28 days simulated microgravity)</th>
<th>Initial</th>
<th>Final</th>
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<tbody>
<tr>
<td>Con 220.4±8.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sus 215.3±9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sus + STD1 207.4±9.6</td>
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respective Con rats \((P < 0.01)\). However, femur length showed no significant differences among different groups in the two experiments. STD for 1 h/day significantly attenuated muscle atrophy in the 3-day experiment and moderately attenuated atrophy in the 28-day experiment: wet weight of 28-day Sus \(\text{STD}_1\) soleus was 34\% less than that of 28-day Con soleus \((P < 0.01)\) (48).

### Table 3. Boltzmann fit parameters for Con, Sus, and Sus \(\text{STD}_1\) cerebral and mesenteric VSMCs

<table>
<thead>
<tr>
<th>Experiment 1 (3 days simulated microgravity)</th>
<th>Cerebral VSMCs</th>
<th>Activation Curves</th>
</tr>
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<tbody>
<tr>
<td>Con</td>
<td>(-8.7 \pm 0.9) (28)</td>
<td>(-1.2 \pm 0.6) (46)</td>
</tr>
<tr>
<td>Sus</td>
<td>(-10.6 \pm 1.2) (21)</td>
<td>(-0.8 \pm 1.7) (36)</td>
</tr>
<tr>
<td>Sus (\text{STD}_1)</td>
<td>(-9.5 \pm 0.7) (24)</td>
<td>(-2.5 \pm 1.1) (34)</td>
</tr>
<tr>
<td>Mesenteric VSMCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>(-9.8 \pm 0.9) (26)</td>
<td>(-1.0 \pm 0.6) (28)</td>
</tr>
<tr>
<td>Sus</td>
<td>(-8.6 \pm 0.7) (23)</td>
<td>(-0.7 \pm 1.2) (26)</td>
</tr>
<tr>
<td>Sus (\text{STD}_1)</td>
<td>(-8.8 \pm 0.7) (25)</td>
<td>(-2.1 \pm 1.3) (28)</td>
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<tbody>
<tr>
<td>Con</td>
<td>(-15.6 \pm 1.5) (17)</td>
<td>(-4.5 \pm 0.8) (22)</td>
</tr>
<tr>
<td>Sus</td>
<td>(-17.6 \pm 1.2) (13)</td>
<td>(-3.7 \pm 0.6) (17)</td>
</tr>
<tr>
<td>Sus (\text{STD}_1)</td>
<td>(-15.9 \pm 1.6) (16)</td>
<td>(-5.3 \pm 1.1) (19)</td>
</tr>
<tr>
<td>Mesenteric VSMCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>(-17.8 \pm 1.3) (18)</td>
<td>(-5.4 \pm 0.7) (24)</td>
</tr>
<tr>
<td>Sus</td>
<td>(-16.6 \pm 1.1) (15)</td>
<td>(-4.8 \pm 0.8) (23)</td>
</tr>
<tr>
<td>Sus (\text{STD}_1)</td>
<td>(-16.9 \pm 1.2) (22)</td>
<td>(-4.3 \pm 0.8) (25)</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE of number of cells in parentheses. \(V_h\), half-maximal voltage; \(k\), steepness factor; VSMCs, vascular smooth muscle cells.

### Ca\(_{\text{L}}\) Current Properties and Densities

#### 3-Day simulation experiment. Whole cell Ca\(_{\text{L}}\) currents recorded in cerebral arterial VSMCs isolated from Con, Sus, and Sus \(\text{STD}_1\) rats are illustrated in Fig. 1, A–C. These families of inward currents were elicited by incremental 10-mV depolarizing steps from a constant holding potential of \(-40\) mV to

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**Fig. 2.** Whole cell Ca\(_{\text{L}}\) currents in mesenteric arterial VSMCs of Con (A), Sus (3 days; B), and Sus \(\text{STD}_1\) (C). Currents were sensitive to 0.1 \(\mu\)M nifedipine. D: \(I-V\) relationships comparing peak Ca\(_{\text{L}}\) current densities among VSMCs from Con, Sus, and Sus \(\text{STD}_1\). Densities were significantly depressed in Sus compared with Con \((\ast P < 0.05)\) and Sus \(\text{STD}_1\) \((\#P < 0.05)\). E: analysis of normalized peak Ca\(_{\text{L}}\) current densities reveals overlapping \(I-V\) relationships, implying similar activation and sensitivity voltages for Ca\(_{\text{L}}\) in Con, Sus, and Sus \(\text{STD}_1\). Sample sizes for Con, Sus, and Sus \(\text{STD}_1\) were 28, 26, and 28, respectively.
test voltages as positive as +60 mV. Nifedipine (0.1 μM) blocked the inward currents, verifying that the inward currents recorded were Ba$^{2+}$ currents through Ca$_L$. The inward currents were larger at all command potentials in the Sus and Sus + STD$_1$ than in the Con myocyte. I-V relationships for VSMCs from Con, Sus, and Sus + STD$_1$ rats ($n = 46, 36, 34$, respectively) are depicted in Fig. 1D. The Ca$_L$ current densities of the cerebral VSMCs from Sus and Sus + STD$_1$ were comparable, and both were significantly larger than those from Con rats ($P < 0.01$). The mean peak Ca$_L$ current densities at +10 mV in Con, Sus, and Sus + STD$_1$ rats were $-4.19 \pm 0.28$, $-5.21 \pm 0.46$, and $-5.21 \pm 0.37$ pA/pF, respectively (see Fig. 7). $C_m$ was not significantly different among Con, Sus, and Sus + STD$_1$ myocytes, averaging $21.7 \pm 0.7, 22.2 \pm 0.8$, and $20.7 \pm 0.6$ pF, respectively. Overlapping of the normalized I-V curves obtained by plotting the Ca$_L$ current density obtained at each test potential as a percentage of the maximal inward current in each cerebrovascular myocyte (20) (Fig. 1E) implies a similar Ca$_L$ activation among Con, Sus, and Sus + STD$_1$ rats. Subsequently, data were fitted to the Boltzmann function as follows: $P = 1/(1 + \exp[(V_h - V)/k])$ and $P = 1/(1 + \exp[(V - V_h)/k])$, where $V$ is the command potential, $V_h$ is the potential for half-maximal activation, and $k$ is a steepness factor (i.e., Boltzmann coefficient). No significant differences were noted in $V_h$ and $k$ among Con, Sus, and Sus + STD$_1$ myocytes (Table 3).

Typical records of whole cell Ca$_L$ currents recorded in mesenteric arterial VSMCs isolated from Con, Sus, and Sus + STD$_1$ rats are shown in Fig. 2, A-C. These inward currents were also blocked by 0.1 μM nifedipine. The inward Ca$_L$ currents were smaller at all command potentials in Sus than in Con myocytes. However, the inward currents in Sus + STD$_1$ myocytes were comparable to those in Con myocytes. The I-V curves of Ca$_L$ currents in mesenteric VSMCs from Con, Sus, and Sus + STD$_1$ were comparable, and both were significantly larger than those from Sus rats ($P < 0.05$). Peak Ca$_L$ current densities at +10 mV in myocytes from Con, Sus, and Sus + STD$_1$ were $-6.09 \pm 0.61$, $-4.42 \pm 0.51$, and $-5.81 \pm 0.53$ pA/pF, respectively (see Fig. 7). The overlapping of the normalized I-V curves of mesenteric VSMCs isolated from Con, Sus, and Sus + STD$_1$ rats is shown in Fig. 2E. Fitted by the Boltzmann function, the values for $V_h$ and $k$ were comparable among Con, Sus, and Sus + STD$_1$ myocytes (Table 3). $C_m$ was not significantly different among Con, Sus, and Sus + STD$_1$ myocytes, averaging $19.5 \pm 0.6, 19.7 \pm 1.4$, and $19.4 \pm 0.8$ pF, respectively.

28-Day simulation experiment. Typical records of whole cell Ca$_L$ currents recorded in cerebral VSMCs in Fig. 3, A–C, show augmented Ca$_L$ currents in cerebrovascular myocytes isolated from a Sus and a Sus + STD$_1$ rat compared with a Con rat. I-V curves in Fig. 3D indicate that the current densities are comparable in the Sus and the Sus + STD$_1$ rat, and both were significantly larger than in the Con rat ($P < 0.01$). Peak Ca$_L$ current densities at +10 mV in cerebrovascular myocytes from Con, Sus, and Sus + STD$_1$ rats were $-5.8 \pm 0.5 (n = 22)$, $-8.3 \pm 0.5 (n = 17)$, and $-8.4 \pm 0.5 (n = 19)$ pA/pF, respectively (see Fig. 7). The normalized I-V curves for Con, Sus, and Sus + STD$_1$ are shown in Fig. 3D. The normalizations were performed by 10 mV in myocytes from Con, Sus, and Sus + STD$_1$ rats, respectively (see Fig. 7). The normalized I-V curves for Con, Sus, and Sus + STD$_1$ are shown in Fig. 3D.
Sus, and Sus + STD1 myocytes overlapped (Fig. 3E). Fitted by the Boltzmann function, the values for \( V_h \) and \( k \) were comparable among Con, Sus, and Sus + STD1 myocytes (Table 3). \( C_m \) showed no significant difference among Con, Sus, and Sus + STD1, averaging 22.8 ± 0.8, 24.7 ± 0.7, and 23.3 ± 0.6 pF, respectively.

Typical records and \( I-V \) curves for mesenteric VSMCs are presented in Fig. 4. \( \text{Ca}_L \) inward currents were drastically depressed in Sus compared with Con myocytes (Fig. 4, A and B); however, these changes were only partially alleviated in the Sus + STD1 myocytes (Fig. 4C). The \( I-V \) relationship in Fig. 4D further reveals partial alleviation of the effect in Sus + STD1 myocytes. Peak \( \text{Ca}_L \) current densities at +10 mV in mesenteric vascular myocytes from Con, Sus, and Sus + STD1 rats were –8.5 ± 0.6 (n = 24), –4.8 ± 0.4 (n = 23), and –6.6 ± 0.5 (n = 25) pA/pF, respectively (see Fig. 7). The normalized \( I-V \) curves do not overlap at +50 and +60 mV (Fig. 4E). \( C_m \) did not significantly change, averaging 25.1 ± 0.7, 23.7 ± 0.9, and 23.0 ± 0.7 pF in Con, Sus, and Sus + STD1 myocytes, respectively. Fitted by the Boltzmann function, the values for \( V_h \) and \( k \) were comparable among Con, Sus, and Sus + STD1 myocytes (Table 3).

**Ca\(_L\) Channel \( \alpha_{1C} \)-Subunit Expression**

3-Day Simulation Experiment. Figure 5, A and B, shows 200- and 240-kDa doublet bands of the \( \alpha_{1C} \)-subunit protein in membranes from cerebral and mesenteric arterial myocytes. These doublet bands correspond to the predicted size of the short and long (or full-length) forms of the \( \text{Ca}_L \) \( \alpha_{1C} \)-subunit protein (20, 29, 30, 34). The similar immunodensity of the \( \beta \)-actin (42 kDa) internal standard in different lanes verified uniform lane loading with membrane proteins (21). Densities of the doublet immunoreactive bands normalized by \( \beta \)-actin did not show significant differences among Con, Sus, and Sus + STD1 cerebral and mesenteric arterial membrane proteins (Fig. 5, C and D).

28-Day Simulation Experiment. Figure 6, A and B, shows 200- and 240-kDa doublet bands of the \( \alpha_{1C} \)-subunit protein in the cerebral and mesenteric arteries. The density of the doublet immunoreactive bands was strikingly increased in cerebral VSMCs and reduced in mesenteric VSMCs of Sus compared with respective Con rats. However, it seems that the differential changes in density were alleviated in the two kinds of myocytes from Sus + STD1 compared with respective Sus rats. In the same lanes, \( \beta \)-actin showed a similar signal density, demonstrating uniformity of lane loading. Averages from four to five separate trials in Fig. 6, C and D, indicate that the density of the immunoreactive doublet bands (expressed as percentage of the \( \beta \)-actin signal) was 121% greater \((P < 0.01)\) or 23% less \((P < 0.05)\) in the cerebral and mesenteric arteries, respectively, of Sus than Con rats. STD for 1 h/day did not prevent the increase in protein expression in cerebrovascular myocytes, and the density was still 75% greater \((P < 0.05)\) in Sus + STD1 than in respective Con rats. However, the reduction of protein expression in mesenteric arterial myocytes was prevented by this intervention, as indicated by a nonsignificant 9% decrease \((P > 0.05)\) in intensity in Sus + STD1 compared with respective Con rats.

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**Figure 4.** Whole cell \( \text{Ca}_L \) currents in mesenteric arterial VSMCs of Con (A), Sus (28 days; B), and Sus + STD1 (C). Currents were sensitive to 0.1 \( \mu \)M nifedipine. D: I-V relationships comparing peak \( \text{Ca}_L \) current densities among VSMCs of Con, Sus, and Sus + STD1. Densities were significantly depressed in Sus \(*P < 0.01\) and Sus + STD1 \(*\&P < 0.05\) compared with Con. There is also a significant difference between Sus and Sus + STD1 \(*&P < 0.05\). E: analysis of normalized peak \( \text{Ca}_L \) current densities reveals overlapping I-V relationships, implying similar activation and sensitivity voltages for \( \text{Ca}_L \) in Con, Sus, and Sus + STD1. Sample sizes for Con, Sus, and Sus + STD1 were 24, 23, and 25, respectively.

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DISCUSSION

The two principal new findings of this study are as follows. 1) Simulated microgravity up- and downregulates CaL current in VSMCs isolated from rat cerebral and mesenteric arteries, respectively. STD for 1 h/day during simulated microgravity for 3 and 28 days can completely (within 3 days) and partially (within 28 days) prevent the reduction of CaL current in mesenteric VSMCs, but it cannot prevent the augmentation of the current observed in cerebrovascular myocytes. 2) Simulated microgravity for 28 days, but not 3 days, can up- and downregulate expression of the α1c-subunit of the CaL channel in VSMCs isolated from rat cerebral and mesenteric arteries. Nevertheless, STD for 1 h/day over 28 days showed differential counteracting effects on protein expression: it prevented the decrease in expression in mesenteric VSMCs but not the increase in expression in cerebral arterial myocytes.

We interpret these new findings in the light of recent advances in vascular biology and gravitational cardiovascular physiology to suggest the important role of channel remodeling in VSMCs during vascular adaptation to microgravity. Furthermore, these findings also provide some new insight into the understanding of the mechanisms underlying the countermeasure effectiveness of IAG on post-bed-rest/postflight cardiovascular deconditioning.

Important Role of Channel Remodeling in VSMCs During Vascular Adaptation to Microgravity

Differential adaptation of cerebral and lower/hindbody vessels during real/simulated microgravity has been postulated to be a problem of vascular autoregulation in response to sustained elevation and reduction of local transmural pressures (15, 39, 45). During head-down tilt or microgravity exposure, the primary change in the vascular system is redistribution of transmural pressures across the vasculature, and blood volume redistribution due to high compliance of the venous system is a secondary consequence. The transmural pressure redistribution is maintained as long as the head-down tilt or microgravity exposure is continued, even though the blood volume redistribution has attained a new equilibrium.

The myogenic tone of small arteries and arterioles varies according to the prevailing intravascular pressures. If blood pressure remains high, the myogenic response may amplify the initial change in blood pressure by increasing vascular tone and may ultimately lead to structural remodeling of vessels (8). Hypotension leads to the opposite changes in vessels. Ex vivo studies with aortic organ culture have shown that a certain level of stretch due to transmural pressure appears to be essential in maintaining vascular smooth muscle components. These studies have demonstrated further that overstretching triggers adaptational processes, resulting in hypertrophy, whereas abnormally lowered transmural pressure results in atrophic changes (19). Cytoplasmic Ca2+ concentration is the most important signal transduction element in maintaining myogenic tone, triggering cell contraction, and regulating growth and/or proliferation of VSMCs (14, 16). Therefore, when blood pressure is at a sustained high level, interactions among CaL, large-conductance Ca2+- and voltage-activated K+ channel, and voltage-gated K+ channel in arterial VSMCs may be disturbed.

Fig. 5. Expression of α1c-subunit in cerebral (A) and mesenteric (B) arterial smooth muscle membranes from Con (lane 1), Sus (3 days; lane 2), and Sus + STD (lane 3). Density of 200- and 240-kDa doublet bands corresponds to short and long forms of α1c-subunit. Expression of β-actin internal standard (42 kDa) was uniform. C and D: averaged data from Western blots in A and B, respectively (n = 4). NS, not significant.
and a new equilibrium will be achieved. Increased expression of the CaL protein and/or increased open-state probability of the CaL channel have been suggested to be responsible for the functional upregulation of CaL (6, 30, 34).

In our previous studies, we demonstrated that 28 days of simulated microgravity increased CaL current in cerebral vascular myocytes comparable with that in myocytes from spontaneously hypertensive rats (SHR) (44). The experiments described here have demonstrated, for the first time, that simulated microgravity for 28 days may result in differential regulation of CaL current and protein expression in VSMCs from cerebral and mesenteric arteries, whereas simulated microgravity for 3 days induces differential changes only in CaL current in the two different kinds of myocytes. The pore-forming protein of the CaL in VSMCs is the α1c-subunit, which represents a splice variant (α1c-b) of the cardiac α1c-a gene (6, 30, 34). The first direct evidence of α1c-subunit mRNA and protein upregulation in adult SHR mesenteric arteries was reported in 2002 by Pratt et al. (30). More recently, direct evidence has indicated that high blood pressure upregulates CaL current in small rat renal arteries by promoting α1c-subunit overexpression and that pressure-induced depolarization of VSMCs might be the potential trigger signal (29, 34).

Furthermore, we report, for the first time, α1c-subunit protein expression in rat cerebral arteries (Table 1 in Ref. 34). Our finding that 3 days of simulated microgravity did not result in significant changes in CaL expression in VSMCs is consistent with the suggestion that overexpression of arterial CaL may be a later event in the development of hypertension in SHR (30, 34). However, Pesic et al. (29) detected overexpression of CaL in right renal arteries exposed to high blood pressure as early as 2 days after aortic banding. This discrepancy might be explained by an immediate greater pressure increase in the right renal artery after banding, in which the mean systolic pressure difference across the banded site was ~80 mmHg. Even in this situation, only 70% of the experimental animals showed a clear result (29). Finally, we observed no significant changes in CaL activation and deactivation dynamics in VSMCs from rats subjected to 3 and 28 days of simulated microgravity. These properties of macroscopic CaL currents also did not show significant changes in hypertensive rats (27, 40). Thus whether alterations in single-channel properties or channel availability could account for the macroscopic changes in CaL current due to simulated microgravity remains to be elucidated. However, in genetically (28) and nongenetically (33) hypertensive rats, the enhanced whole cell current in arterial VSMCs has been attributed to the increased opening of single CaL channels, not to changes in their properties.
Briefly, our data support the notion that respective changes in function and protein expression of CaL in different arterial VSMCs were induced by sustained elevation and reduction of local transmural pressures in cerebral and hindquarter arteries during simulated microgravity. We have further speculated that the vascular pressure-dependent polarization state of the membranes of VSMCs might be among the potential signals that trigger the adaptations in function and protein expression of vascular CaL (29, 34).

Remodeled CaL Channels in Cerebral and Mesenteric VSMCs Respond Differently to Daily Short-Duration STD During Simulated Microgravity

The results of the intervention experiments were contrary to our expectation that the countermeasure would prevent the effects of simulated microgravity on CaL current and expression in different kinds of VSMCs.

For cerebral vessels, STD for 1 h/day did not show any counteracting effect in preventing the augmentation of CaL function and protein expression during short-term (3 days) and medium-term (28 days) simulated microgravity (Figs. 6, A and C, and 7). The unresponsiveness of the CaL channel in cerebral arterial VSMCs to such an intervention seems to be an important mechanism to ensure an increased Ca2+/H1001 influx for the maintenance of an increased myogenic tone whenever the rat is subjected to simulated microgravity. The enhanced Ca2+/H1001-dependent vascular tone is an important protective mechanism against an elevated cerebral perfusion pressure induced by simulated microgravity to reduce the risk of excessive capillary filtration, cerebral edema, and possible stroke (12, 15, 39, 45). Although it has been shown that simulated microgravity increases myogenic tone (12, 41, 42), enhances receptor- and non-receptor-mediated vasoconstrictor responsiveness (49), and results in hypertrophic remodeling of the cerebral vessels (25, 43, 45) and that daily 1-h −Gx by STD is sufficient to prevent the vasoreactivity and remodeling changes (35, 46), whether the increased tone can also be prevented by such a countermeasure remains unknown. These findings seem to imply that the pressure-induced and CaL-mediated myogenic tone response can be dissociated from other functional and structural adaptations in the cerebrovascular wall during microgravity exposure.

For mesenteric arterial VSMCs, STD for 1 h/day is effective in preventing CaL current decrement during 3 days of simulated microgravity; however, it becomes only partially effective when the simulation period is prolonged to 28 days (Fig. 7). Moreover, the reduction of protein expression might also be prevented by such an intervention over a 28-day period (Fig. 6, B and D). The responsiveness of CaL in mesenteric VSMCs to this intervention is consistent in general with our previous findings that STD for 1 h/day can prevent the depression in vasoconstrictor responsiveness and atrophic changes that may occur in hindlimb vessels due to simulated microgravity alone (35). It also provides a mechanistic explanation for the potential benefit of IAG in preventing postspacelift cardiovascular deconditioning (4, 5, 32, 36–38, 46). Inability to adequately elevate the total peripheral resistance has been identified as an important factor in the genesis of postflight orthostatic intolerance (1, 2, 39, 45), whereas splanchnic and muscular vascular beds are the main contributors to the maintenance of peripheral resistance. Thus our findings have provided evidence to suggest that the potential benefit of IAG might stem from its modulatory effect on vascular channel remodeling in vascular myocytes of resistance vessels. Nevertheless, whether IAG is efficacious during prolonged exposure to microgravity or whether a longer exposure to IAG is needed remains unknown, since the duration of most of the human studies was <13 days (5, 32, 36–38), whereas the animal studies lasted for 28 days (35, 46, 48). The present study has further demonstrated that prevention of CaL current decrement in mesenteric arterial myocytes is incomplete when the simulated microgravity is extended to 28 days. Two possibilities merit further consideration. 1) Channel remodeling in the membrane structure of mesenteric VSMCs might be a dynamic process during vascular adaptation to simulated microgravity (6, 34). At 4 wk, the partially restored CaL function could be enough to cope...
with the requirement for $\text{Ca}^{2+}$ influx, since the vascular remodeling in mesenteric arteries due to simulated microgravity alone would have been completely prevented by the intervention of daily 1-h exposure to $-G_{\text{K}}$ (35, 46). 2) Different $\text{Ca}^{2+}$ channels, or $\text{Ca}_{\text{L}}$ subunits, are affected during the adaptation with the intervention that produces altered whole cell electrophysiological profiles.

Study Limitations and Perspectives

1) Single-channel properties of $\text{Ca}_{\text{L}}$ were not included for detailed analysis of channel properties and kinetics. 2) Whether STD for 1 h/day could influence the regulation of myogenic tone of cerebral (12) and small mesenteric (23) arteries during simulated microgravity was not examined. Thus it remains unclear whether regulation of myogenic tone and regulation of vasoconstrictor responsiveness were dissociable. 3) It is a great challenge to elucidate the complexity of the mechanisms underlying the high responsiveness of vessels to the intervention of daily short-duration gravitational loading during microgravity exposure. Among the many factors contributing to individualities of vascular function in different organ systems, the present study addressed only the $\text{Ca}_{\text{L}}$ channel mechanism. It is also important to extend the study to alterations in channel remodeling and second-messenger function. For example, endothelial nitric oxide synthase signaling (42) and the local renin-angiotensin system (46, 47) are involved in cerebrovascular adaptation during simulated microgravity. Studies have suggested that angiotensin infusion-induced $\text{Ca}_{\text{L}}$ current increase might be related to mislocalization of endothelial nitric oxide synthase (13). Additional work is also required to elucidate whether the $\text{Ca}_{\text{L}}$ current is altered via second-messenger mechanisms and whether different $\text{Ca}^{2+}$ channels or $\text{Ca}_{\text{L}}$ subunits are affected in rats subjected to simulated microgravity with or without interventions.

In conclusion, we have shown that, in response to short-term (3 days) and medium-term (28 days) simulated microgravity, $\text{Ca}_{\text{L}}$ current increases in cerebral and decreases in mesenteric arterial VSMCs of rats, and, correspondingly, differential regulations in protein expression of the $\text{Ca}_{\text{L}}$ channel $\alpha_{\text{Lc}}$-subunit occur after a medium-term simulation. However, the intervention of daily 1-h $-G_{\text{K}}$ by STD has different effects on $\text{Ca}_{\text{L}}$ current and protein expression in different artery types. 1) In mesenteric arterial VSMCs, it can prevent the decrease of $\text{Ca}_{\text{L}}$ current during a short-term exposure and alleviate the decrease in current and prevent the reduction of $\alpha_{\text{Lc}}$-subunit expression during a medium-term exposure. 2) In cerebrovascular myocytes, the augmented current and increased expression of $\text{Ca}_{\text{L}}$ that would occur due to simulated microgravity alone are not prevented by such an intervention.

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GRANTS

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