Simulated microgravity alters rat mesenteric artery vasoconstrictor dynamics through an intracellular Ca\(^{2+}\) release mechanism

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Colleran PN, Behnke BJ, Wilkerson MK, Donato AJ, Delp MD. Simulated microgravity alters rat mesenteric artery vasoconstrictor dynamics through an intracellular Ca\(^{2+}\) release mechanism. Am J Physiol Regul Integr Comp Physiol 294: R1577–R1585, 2008. First published March 19, 2008; doi:10.1152/ajpregu.00084.2008. —Previous work has shown that orthostatic hypotension associated with cardiovascular deconditioning results from inadequate peripheral vasoconstriction. We used the hindlimb-unloaded (HU) rat in this study as a model to induce cardiovascular deconditioning. The purpose of this study was to test the hypothesis that 14 days of HU diminishes vasoconstrictor responsiveness of mesenteric resistance arteries. Mesenteric resistance arteries from control (n = 43) and HU (n = 44) rats were isolated, cannulated, and pressurized to 108 cm H\(_2\)O for in vitro experimentation. Myogenic (intralumenal pressure ranging from 30 to 180 cm H\(_2\)O), KCl (2–100 mM), norepinephrine (NE, 10\(^{-9}\)–10\(^{-4}\) M) and caffeine (1–20 mM) induced vasoconstriction, as well as the temporal dynamics of vasoconstriction to NE, were determined. The active myogenic and passive pressure responses were unaltered by HU when pressures remained within physiological range. However, vasoconstrictor responses to KCl, NE, and caffeine were diminished by HU, as well as the rate of constriction to NE (C, 14.8 ± 3.6 μm/s vs. HU 7.6 ± 1.8 μm/s). Expression of sarcoplasmic reticulum Ca\(^{2+}\)ATPase 2 and ryanodine 3 receptor mRNA were unaffected by HU, while ryanodine 2 receptor mRNA and protein expression were diminished in mesenteric arteries from HU rats. These data suggest that HU-induced and microgravity-associated orthostatic intolerance may be due, in part, to an attenuated vasoconstrictor responsiveness of mesenteric resistance arteries resulting from a diminished ryanodine 2 receptor Ca\(^{2+}\) release mechanism.

Hindlimb unloading; vasoconstrictor responsiveness

Following exposure to weightlessness, many astronauts experience postflight orthostatic hypotension (6, 52) due, in part, to a diminished ability of the cardiovascular system to rapidly elevate peripheral vascular resistance (PVR) (1, 2, 8, 36, 52, 53). It has been proposed that the diminished ability to elevate PVR following spaceflight may result from altered function of the afferent limb of the baroreceptors, central integration, sympathetic efferents, or end-organ responsiveness to sympathetic stimulation (8). Evidence for end-organ (vascular) hyporesponsiveness has been provided by Whitson et al. (54) and Levine et al. (27), who reported no difference in preflight vs. postflight PVR during orthostasis despite higher postflight levels of plasma catecholamine concentration and muscle sympathetic nerve activity. Similar reductions in vasoconstrictor responsiveness have been reported by investigators using prolonged bedrest (44, 46). Therefore, evidence suggests end-organ responsiveness to vasoconstrictor stimuli is diminished in humans following microgravity and bedrest deconditioning. In addition, orthostatic intolerance may reflect the relative inability of arteries to rapidly vasoconstrict. Specifically, a reduced rate of arterial constriction in modulating PVR in response to an orthostatic challenge could contribute to a compromised ability to precisely regulate mean arterial pressure.

The tail-suspended hindlimb unloaded (HU) rat simulates a weightless environment and bedrest deconditioning through the mechanical unloading of the hindlimb bones and muscles and the associated cephalic fluid shift (17, 55). HU rats demonstrate many cardiovascular adaptations characteristic of cardiovascular deconditioning in humans, including a reduced exercise capacity (17, 39), orthostatic hypotension (29, 55), and a diminished capacity to elevate PVR (31, 57). This diminished ability to elevate PVR has been shown to be related to reductions in vasoconstrictor responsiveness of a number of conduit arterial segments (14, 16, 40, 41, 43, 59) and skeletal muscle arterioles (13) from HU rats. However, to date, there have been no investigations that determine whether the rate of arterial vasoconstriction is altered by hindlimb unloading, only whether the magnitude and sensitivity of vasoconstriction is affected. Therefore, the purpose of the present study was to 1) determine whether hindlimb unloading diminishes the rate of vasoconstriction of mesenteric arteries to NE, and 2) examine vasoconstrictor responses working through a variety of cell signaling pathways (i.e., myogenic, NE, KCl, and caffeine) to determine a possible mechanism for the reported decrement in mesenteric vasoconstriction in HU rats and whether it is related to a diminished sarcoplasmic reticulum intracellular Ca\(^{2+}\) release mechanism. We hypothesized that 14 days of hindlimb unloading would diminish the rate of mesenteric artery vaso-
constriction, as well as reduce the vasoconstrictor response to each protocol tested.

MATERIALS AND METHODS

All procedures performed in this study were approved by the Texas A&M and West Virginia University Animal Care Committees and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals

Male Sprague-Dawley rats weighing 438 ± 7 g were obtained (Harlan, Indianapolis, IN) and housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. The animals were randomly assigned to either cage control (n = 43) or 14 day HU (n = 44) groups. The hindlimbs of the HU group were elevated to an approximate spinal angle of 40–45° via orthopedic tape adhered to the tail (5, 15, 56), which is a modification of techniques previously described (58). Control animals were individually housed and maintained in a normal cage environment, while HU rats were kept in the head-down position for 14 days. This time period has been shown to be sufficient to induce cephalic fluid shifts (30, 55) and produce cardiovascular alterations in HU animals (13, 17, 29, 31, 38, 39, 55, 57). After the experimental period, HU and control animals were injected with pentobarbital sodium (60 mg/kg ip) to induce deep anesthesia without allowing the hindlimbs of the HU rats to become weight bearing. The animals were then weighed, decapitated, and the gastrointestinal tract and soleus muscle were carefully dissected free and weighed; the gastrointestinal tract was placed in a 4°C filtered physiological saline solution (PSS) (pH 7.4) for mesenteric artery isolation.

Microvessel Preparation

Under a dissecting microscope, distal arcading resistance arteries of the mesentery (~200 μm), immediately proximal to the transmural arterioles, were identified, dissected free from surrounding tissue, transferred to a Lucite vessel chamber containing PSS-albumin solution (pH 7.4), and cannulated as previously described (5). The mesenteric artery segments were then pressurized at 108 cm H2O, which corresponds to in vivo arterial pressures for these vessels (21). The vessels were allowed to equilibrate for 1 h at 37°C before intrinsic vasomotor properties were characterized. Internal diameters were measured continuously throughout the experiment using the video recorder.

Experimental Design

Protocol I: myogenic vasoconstrictor responses. Active myogenic responsiveness to step changes in intralumenal pressure was characterized in the initial group of mesenteric arteries. Following the equilibration period at baseline pressures, intralumenal pressure was incrementally increased from 110 to 180 cm H2O by 10 cm H2O, reduced incrementally to 30 cm H2O, and finally increased back up to baseline pressure in 10 cm H2O increments. Intralumenal pressure and vessel diameter were recorded continuously for 5 min following each incremental change in pressure. A passive pressure-diameter relation was then established by filling the vessel chamber with calcium-free PSS containing 2.0 mM EDTA. The calcium-free PSS was replaced every 15 min over a 60-min period to facilitate relaxation of the vascular smooth muscle before determining the passive pressure-diameter relation. The intralumenal pressure was then incrementally changed as describe above. Diameter was continuously recorded for 3 min following each pressure change. Results from the active myogenic response indicated that when intralumenal pressure was elevated from baseline up to 180 cm H2O, there was no difference in the arterial response between control and HU rats (Fig. 1A), when pressure was lowered to 30 cm H2O, there was no difference (Fig. 1B), but when it was raised from 30 cm H2O back to baseline intralumenal pressure, the myogenic vasoconstriction was less in arteries from HU rats (Fig. 1C). This result was surprising, given that one might have expected that differences in vasoconstriction would be most evident at the highest pressures. Thus, we sought to determine whether this difference in the myogenic response was the result of one of several possible factors: 1) that the vessels from HU rats had become less viable at the end of a rather prolonged test protocol, or 2) whether the exposure to a low intralumenal pressure might have somehow triggered the different response. Therefore, a second series of studies was performed that was of the same duration as the first series, but the pressure was not lowered to subphysiological levels. In this second series, intralumenal pressure was set at 108 cm H2O, and then intralumenal pressure was increased by 10 cm H2O increments up to 180 cm H2O and incrementally reduced to 100 cm H2O. Intralumenal

Fig. 1. Active myogenic response of mesenteric resistance arteries to changes in intralumenal pressure from 108 to 180 cm H2O (A), 180 to 30 cm H2O (B), and 30 to 110 cm H2O (C) in control (Con) and 14-day hindlimb unloaded (HU) animals. Values are expressed as means ± SE. *HU diameter different from that of Con (P < 0.05).
pressure was then maintained at 100 cm H2O for a period of 85 min, which was then followed by a pressure increase to 110 cm H2O. Thus, the duration of this test corresponded to the duration of the previous myogenic response, the only difference being that this pressure-response relation did not expose the mesenteric arteries to an intraluminal pressure lower than 100 cm H2O. Lumenal diameter was continuously recorded for 5 min following each pressure change and during the maintenance period at 100 cm H2O.

Protocol II: KCl- and norepinephrine-mediated vasoconstriction. Concentration-diameter responses to potassium chloride (KCl), which mediates vasoconstriction through voltage-gated Ca2+ channels, and norepinephrine (NE), which mediates vasoconstriction through α-adrenergic receptors, were determined in a third group of animals. Vasoconstrictor properties of mesenteric resistance arteries were characterized by determining maximal responsiveness and sensitivity to NE (10−8 – 10−4 M) and KCl (2 – 100 mM).

Protocol III: caffeine-mediated vasoconstriction. Vasocostrictor responses induced by intracellular Ca2+ release from the sarcoplasmic reticulum were evaluated in mesenteric arteries by establishing a noncumulative concentration-response relation for caffeine (1, 3, 5, 10, 15, and 20 mM) as previously described (9). Peak contractile responses to increasing concentrations of caffeine in calcium-free PSS were recorded at 20-min intervals. Following each application of caffeine, the vessels were washed with 2 mM Ca2+ PSS for 20 min to reestablish intracellular Ca2+ stores.

Protocol IV: vasoconstrictor dynamics. Vasoconstrictor dynamics of mesenteric resistance arteries were characterized by determining the temporal responses to NE. Mesenteric artery images were obtained using a similar setup as above (i.e., inverted microscope, Lucite viewing chamber) and were viewed on a high-resolution monitor (Olympus Trinonitrol OEV143). Images were time referenced by frame and stored on digital media (Maxell Mini DV, DVM60SE) for off-line analysis via digital video recorder (Sony DSR-30 DVR). Following cannulation and the equilibration period at baseline pressure, mesenteric arteries were exposed to 10−4 M NE and subsequent changes in luminal diameter were recorded for off-line analysis. The dose of 10−4 M NE was selected to avoid the possible confounding influence of group differences in arterial sensitivity (EC50) to NE at the lower doses. Changes in mesenteric artery luminal diameter with exposure to NE were observed with frame-by-frame playback (30 frames/s) using a video caliper (Colorado Video, 307A, Boulder, CO) to quantify lumen diameter. Subsequently, the time-diameter values were curve-fit to a monoeponential plus delay model (4) using an iterative least-squares technique by means of a commercial graphing/analysis package (KaleidaGraph 3.5). For the KaleidaGraph analysis program, a user-defined function to the data was fitted using the equation as follows: Diameter(t) = Diameter(b) − ΔDiameterss [1 − e−(t−TD)/τ], where Diameterss is the change in diameter at time t, Diameter(b) is baseline diameter, ΔDiameterss is the change in diameter from baseline to the steady-state value, TD is the time delay, and τ is the time constant of the response, which estimates the time taken to reach 63% of the final exponential response. The same model was applied to diameter responses of mesenteric arteries from control and HU animals. From the mathematical modeling results, the mean response time (MRT; TD + τ) and the rate of vasoconstriction (ΔDiameter/τ) were calculated.

Protocol V: mRNA and protein expression. Mesenteric artery segments from animals used in the above four protocols and of similar arterial size to those used for in vitro experimentation were isolated, snap frozen, and stored at −80°C for mRNA and protein expression as previously described (18, 47). Briefly, for mRNA expression, arteries were pulverized in lysate buffer, and total RNA was extracted with the RNAqueous filter system (Ambion). Total RNA was used to perform real-time PCR with TaqMan Pre-Developed Assay Reagents to quantitatively determine sarcoplasmic reticulum Ca2+ ATPase 2 (product number Rn01545053_gH), ryanodine 2 receptor (RyR2) (product number Rn01740310_mH), and ryanodine 3 receptor (RyR3) (product number Rn01485062_mH) mRNA expression (ABI Prism 7700 Sequence Detection system).

On the basis of results from mRNA analyses, RyR2 protein expression in mesenteric resistance arteries was determined. For Western blot analysis of RyR2 protein expression, mesenteric artery segments were homogenized in lysis buffer (5 min at 4°C) containing 40 mM 3-(Cyclohexylamino)-1-propanesulfonic acid, 1 mM dl-dithiothreitol, 10 mM EDTA, 15 mM MgCl2, 115 mM NaCl, 1 mM Na-orthovanadate, 1 mM NaF, 2.5 mM urea, 0.25% deoxycholate, 10% glycerol, 1% NP-40, 0.2% SDS, and 1:50 mammalian protease inhibitor cocktail (Sigma, St. Louis, MO). Equal amounts of sample protein (10 μg total protein per sample) were separated on a 4–20% gradient polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were divided at the ladder 117 kDa band and exposed to either monoclonal RyR2 antibody (anti-mouse, 2 μg/ml; Calbiochem, San Diego, CA) or GAPDH antibody (anti-mouse, 1 μg/ml; Chemicon Labs, Temecula, CA). Alexa Fluor 680 goat anti-mouse (1:10,000 dilution; Molecular Probes, Carlsbad, CA) was used to fluorescently label the RyR2 and GAPDH antibodies. The density of signals specific for the RyR2 and GAPDH bands in a given lane on a membrane was measured after the membrane was scanned with an Odyssey infrared imaging system (Li-COR Biosciences, Lincoln, NE).

Solutions and Drugs

The PSS buffer contained (in mM) 145 NaCl, 4.7 KCl, 1.2 NaH2PO4, 1.17 MgSO4, 2.0 CaCl2, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS with a pH of 7.4. Ca2+-free PSS buffer was similar to the PSS buffer except in contained 2 mM EDTA, and CaCl2 was replaced with 2.0 mM NaCl. Concentrated stock solutions of KCl and NE were prepared in PSS buffer. Caffeine was dissolved directly into Ca2+-free PSS to yield the desired concentration.

Statistical Analysis

Intraluminal diameters were measured throughout the duration of each experimental protocol. At the end of each experiment, the vessel was placed in Ca2+-free bathing solution and rinsed several times over the course of an hour to obtain a maximal diameter. The development of spontaneous tone was expressed as the percent constrictive relative to maximal diameter, as previously described (13, 35). Pressure-response and concentration-response curves were expressed as intraluminal diameter (μm) and evaluated using repeated-measures ANOVA with one within (intraluminal pressure or concentration) and one between (experimental groups) factor. Planned contrasts were conducted at each intraluminal pressure or concentration level to determine whether differences exist between experimental groups (control vs. HU). To detect differences in sensitivity to vasoconstrictors, EC50 values were designated as the concentration of each substance producing 50% of its maximal response. Student’s unpaired t-test was used to determine whether differences in body mass, soleus muscle mass, soleus muscle-to-body weight ratio, developed spontaneous tone, maximal diameter, and EC50 values were significant. For vasoconstriction dynamics (i.e., protocol IV), luminal diameters and the resultant model parameters within and between groups were analyzed by means of a one-way ANOVA. Individual significant differences were examined post hoc using the Tukey test. All values are presented as means ± SE. A value of P < 0.05 was required for significance.

RESULTS

Soleus Muscle-to-Body Mass Ratio

The body mass of HU rats (430 ± 14 g) was lower than that of control rats (472 ± 17 g). Soleus muscle mass was 42%
lower (127 ± 5 mg) following HU compared with control (222 ± 6 mg). Similarly, HU resulted in a 35% reduction in the soleus muscle-to-body mass ratio (control, 0.46 ± 0.01 mg/g vs. HU, 0.30 ± 0.01 mg/g; \( P < 0.05 \)). Soleus muscle atrophy, which is characteristic of reduced hindlimb skeletal muscle weight-bearing activity, confirms the efficacy of the HU treatment.

**Vessel Characteristics**

The maximal intraluminal diameter (determined in \( \text{Ca}^{2+} \)-free solution) of mesenteric resistance arteries did not differ between control (237 ± 16 \( \mu \)m) and HU (253 ± 6 \( \mu \)m) rats. Likewise, HU had no effect on the development of spontaneous tone (control, 42 ± 2%; HU, 33 ± 5%).

**Myogenic Response**

Hindlimb unloading had no effect on myogenic responses to pressure increases from 108 to 180 cm H\(_2\)O (Fig. 1A) or to reductions in pressure from 180 to 30 cm H\(_2\)O (Fig. 1B). However, myogenic vasoconstriction to pressure increases from 30 to 110 cm H\(_2\)O was less in mesenteric arterioles from HU rats compared with those of control vessels (Fig. 1C). Myogenic properties of mesenteric arteries exposed to the second pressure response protocol, that is, those not exposed to intraluminal pressures below 100 cm H\(_2\)O, were not affected by HU (Fig. 2A). The findings of the active myogenic response did not differ when the data were analyzed as actual diameters, as shown in the figures, or as percent constriction. Hindlimb unloading had no effect on passive pressure-response characteristics (Fig 2B).

**Responses to NE, KCl, and Caffeine**

Increases in NE and KCl concentration produced dose-dependent decreases in intraluminal diameter in mesenteric arteries. Both the maximal response and the sensitivity (EC\(_{50}\)) of mesenteric arteries to NE were significantly diminished following HU (Fig. 3A). Vasoconstrictor responsiveness to KCl was lower in mesenteric arteries from HU rats (Fig. 3B); the maximal vasoconstrictor response to KCl was reduced by HU, while sensitivity was not altered. Vasoconstrictor responses to caffeine were lower in mesenteric arteries from HU rats (Fig. 4). These findings with NE, KCl, and caffeine did not differ regardless of whether the data are expressed as actual diameters or as a percent constriction.

**Vasoconstriction Dynamics**

The model chosen (i.e., delay followed by exponential) provided a superb fit to the data evidenced by the low \( \chi^2 \) values (control, 28.9 ± 6.1; HU, 45.6 ± 8.3) and high correlation coefficient (control, \( r = 0.978 \pm 0.028 \); HU, 0.958 ± 0.019). Neither the initial diameter nor maximal diameter was different between groups (Table 1). As demonstrated in Fig. 5, upon exposure to 10\(^{-4}\) M NE, mesenteric arteries from both groups displayed a short latent period (time-delay) where luminal diameter did not change, followed by a monoeponential decline to a steady lumen diameter, with a marked blunting in the dynamics of vasoconstriction in HU. The time-delay (TD) preceding the monoeponential decline from HU mesenteric arteries was over twice as long vs. control (Table 1), and the time constant (\( \tau \)) of the exponential decline was significantly slowed in HU vs. control groups (Table 1). The mean response time (TD + \( \tau \)) was ~70% slower in HU vs. control (control, 2.4 ± 0.1 s vs. HU, 4.4 ± 0.3 s; \( P < 0.05 \)). The slowed dynamics in HU resulted in an almost doubling of the time taken to reach a steady-state lumen diameter vs. control (Fig. 6B). HU also demonstrated a smaller magnitude of luminal diameter change to NE (\( \Delta \), control, 132 ± 25 \( \mu \)m vs. HU, 74 ± 15 \( \mu \)m; \( P < 0.05 \); consistent with a reduced sensitivity and maximal response as discussed above), which, when coupled with the slower time constant, result in a reduced rate of contraction in arteries from HU rats (Fig. 6A).

**mRNA and Protein Expression**

Sarcoplasmic reticulum \( \text{Ca}^{2+} \) ATPase 2 (control, 12 ± 3 \( \text{Ca}^{2+} \) ATPase 2:18s; HU, 15 ± 3 ATPase 2:18s) and RyR3 (control, 10 ± 3 RyR3:18s; HU, 20 ± 10 RyR3:18s) mRNA expression did not differ between groups, while no mRNA expression for RyR1 was detected in mesenteric arteries for either group (control, \( n = 10 \); HU, \( n = 12 \)). In contrast, both
RyR2 mRNA (Fig. 7) and protein (Fig. 8) expression were lower in mesenteric arteries from HU rats.

DISCUSSION

The purpose of this study was to determine whether hindlimb unloading alters the intrinsic vasoconstrictor responsiveness of mesenteric resistance arteries. The data demonstrate that hindlimb unloading diminishes vasoconstrictor responsiveness, including the rapidity (Figs. 5 and 6), sensitivity (Fig. 3A), and maximal response (Figs. 3, A and B) of mesenteric arteries to the constrictor agonists NE and KCl. Further, constriction mediated through a sarcoplasmic reticulum Ca\(^{2+}\)/H\(_{1001}\) release mechanism was diminished with hindlimb unloading (Fig. 4), and this was associated with a downregulation of arterial RyR2 mRNA and protein expression (Figs. 7 and 8). The functional consequence of such changes in the vasoconstrictor characteristics of mesenteric arteries could be a compromised ability to elevate vascular resistance, centrally mobilize blood volume, and precisely regulate mean arterial pressure during an orthostatic challenge.

Hindlimb unloading reduced the vasoconstrictor response of mesenteric arteries to caffeine (Fig. 4). Previous work has shown that Ca\(^{2+}\) release from the sarcoplasmic reticulum occurs in response to increasing concentrations of NE, KCl, and caffeine (7, 10, 48). Any alteration in the ryanodine receptor function-Ca\(^{2+}\)/H\(_{1001}\) release mechanisms from the sarcoplasmic reticulum could consequently alter contractile responsiveness of arterial smooth muscle cells to these agonists. The reductions in caffeine-induced vasoconstriction (Fig. 4) and the

![Fig. 4. Concentration-response relation to the noncumulative addition of caffeine in Ca\(^{2+}\)-free buffer solution in mesenteric resistance arteries from control (Con) and 14-day HU rats. Values are expressed as means ± SE. *HU diameter different from that of Con (P < 0.05).](http://ajpregu.physiology.org/content/294/5/R1581/suppl/DC1/Fig4.png)

Table 1. Characteristics and model parameters for the dynamics of vasoconstriction in mesenteric arteries from control and HU animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HU</th>
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<tbody>
<tr>
<td>Maximal diameter, μm</td>
<td>209±16</td>
<td>192±20</td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>19±2</td>
<td>18±1</td>
</tr>
<tr>
<td>Initial diameter, μm</td>
<td>166±22</td>
<td>156±21</td>
</tr>
<tr>
<td>Delta, μm</td>
<td>132±25</td>
<td>74±15*</td>
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<tr>
<td>Time delay, s</td>
<td>0.7±0.1</td>
<td>1.8±0.2*</td>
</tr>
<tr>
<td>Time constant, s</td>
<td>1.7±0.1</td>
<td>2.6±0.2*</td>
</tr>
<tr>
<td>MRT, s</td>
<td>2.4±0.1</td>
<td>4.4±0.3*</td>
</tr>
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</table>

Values are presented as means ± SE; control (n = 8) and hindlimb unloading (HU) (n = 8). Maximal diameter is the luminal diameter measured in Ca\(^{2+}\)-free physiological saline solution; Initial diameter is the lumen diameter measured immediately before application of NE; Delta (Δ), initial diameter minus the steady-state luminal diameter; Time delay (TD), reflects the period when lumen diameter is not changing after exposure to NE; Time constant (τ), reflects the time taken to achieve 63% of the final steady-state value during the exponential fall; MRT, mean response time [(TD + τ), global index of the speed of the response]. *HU value different from that of control (P < 0.05).
downregulation of RyR2 mRNA and protein expression (Figs. 7 and 8), an important ryanodine receptor isoform in smooth muscle cells (24, 25), in the mesenteric arteries with unloading therefore supports the hypothesis that reductions in intracellular Ca\(^{2+}\) release via a RyR2 mechanism could account for the hindlimb unloading-induced reduction in the contractile responsiveness of mesenteric arteries to NE and KCl. This notion is consistent with the observations of Morel et al. (34), who demonstrated that 14-day hindlimb unloading significantly reduced maximal increases in intracellular Ca\(^{2+}\) elicited by caffeine, NE, and ANG II in rat portal vein myocytes. Using \[^3\text{H}\]ryanodine binding, these authors (34) further showed a reduction in the number of ryanodine receptors in the sarcoplasmic reticulum, while ryanodine receptor Ca\(^{2+}\) sensitivity and voltage-gated Ca\(^{2+}\) channel activity remained unaltered by hindlimb unloading. It has also been reported that hindlimb unloading causes a significant increase in sarcoplasmic reticulum Ca\(^{2+}\)-ATPase protein and activity in soleus muscle fibers (45). Increases in vascular smooth muscle cell Ca\(^{2+}\)-ATPase activity and calcium sequestration could further contribute to a lower intracellular Ca\(^{2+}\) concentration during agonist-induced contraction. However, the lack of an increase in mesenteric artery Ca\(^{2+}\)-ATPase 2 mRNA expression suggests that this does not occur in mesenteric smooth muscle cells with unloading.

The myogenic vasoconstrictor results of this present study (Fig. 1C) corroborate those of Loof-Wilson and Gisolfi (28), who report diminished myogenic properties in mesenteric arteries isolated from HU rats, despite several differences in the study design. For example, Loof-Wilson and Gisolfi (28) exposed animals to hindlimb unloading for 28 days, while animals in this present study were unloaded for a period of 14 days, and the pressure-diameter relation was evaluated in the presence of phenylephrine when arterioles failed to develop spontaneous tone, which was not done in the current study. Mesenteric arteries in this present study were exposed to a comparable range of pressures as used by Loof-Wilson and Gisolfi (28); however, the pressure in the present study began at that measured in vivo (108 cm H\(_2\)O or ~80 mmHg) and was incrementally elevated to 180 cm H\(_2\)O, lowered to 30 cm H\(_2\)O, and then elevated back to an in vivo pressure (Fig. 1). There was no difference in myogenic responsiveness between HU arteries and controls within the physiological range of pressures (Figs. 1, A and B). However, only after the arteries were exposed to low pressures was a difference between groups apparent at the higher pressures (Fig. 1C), as was also the case.
for Looft-Wilson and Gisolfi (28). To control for the possibility that the difference in myogenic constriction occurred at the end of a prolonged pressure-diameter response protocol, another time-matched study was performed that did not expose the vessel to low pressures. In this study, there were no differences in myogenic responses between groups (Fig. 2A). These data suggest that myogenic autoregulation within the physiological range of pressures is not altered by HU in mesenteric resistance arteries. Only when the arteries are exposed to low intraluminal pressures does a difference between groups become apparent. The reason(s) underlying this phenomenon remains to be determined but may be related to mobilization of extracellular vs. intracellular Ca\(^{2+}\) at lower and higher transmural pressures.

It is currently believed that myogenic constriction in response to increased intraluminal pressure is initiated by vascular smooth muscle membrane depolarization, which then permits extracellular Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels (12, 32). The dependence on extracellular Ca\(^{2+}\) for contraction and myogenic tone in isolated skeletal muscle (19, 49) and mesenteric (33) resistance arteries has been established. There is some evidence that the myogenic response may also involve release of Ca\(^{2+}\) from intracellular stores (11, 37). However, a number of studies have shown that isolated, cannulated arterioles retain their myogenic responsiveness when release of intracellular Ca\(^{2+}\) from the sarcoplasmic reticulum is blocked (26, 50, 51). Together, these studies appear to indicate that although mobilization of intracellular Ca\(^{2+}\) stores may be involved in the myogenic response, release of intracellular Ca\(^{2+}\) is not essential for myogenic constriction. Hindlimb unloading diminished the mesenteric arteriolar contractile responsiveness to KCl, NE, and caffeine, all of which are involved in the myogenic response, release of intracellular Ca\(^{2+}\) not essential for myogenic constriction. Hindlimb unloading diminished the mesenteric arteriolar contractile responsiveness to KCl, NE, and caffeine, all of which are dependent on the release of intracellular Ca\(^{2+}\) from the sarcoplasmic reticulum (7, 10, 48). Morel et al. (34) reported no effect of hindlimb unloading on voltage-gated Ca\(^{2+}\) channel activity in rat portal vein myocytes. Therefore, if myogenic contraction is primarily dependent on influx of extracellular Ca\(^{2+}\) at pressures within the physiological range (26, 50, 51), then myogenic responsiveness may remain intact despite any unloading-induced alterations in sarcoplasmic reticulum function and RyR2 expression occurring within the mesenteric vasculature. The differential effect of hindlimb unloading on myogenic vs. agonist-induced contractions observed in the present study therefore appears to reflect the different sources of Ca\(^{2+}\) for myogenic and agonist-induced contractions.

The results of the present study indicate that hindlimb unloading diminishes the NE- and KCl-induced vasoconstrictor responses of mesenteric arteries in a manner similar to that observed in the thoracic and abdominal aorta (14, 16, 40, 41) and carotid and femoral arteries (41). As originally proposed by Delp et al. (16), the diminished vasoconstrictor response following HU could result from reductions in intracellular Ca\(^{2+}\) concentration or alterations in the vascular smooth muscle contractile apparatus. In addition to the diminished RyR2-intracellular Ca\(^{2+}\) release mechanism, as described above, there is also experimental support that unloading alters vascular smooth muscle contractile proteins. Recent work indicates that unloading reduces myosin light chain-20 and myosin heavy chain protein expression in rat femoral arteries (23). If such changes in the contractile apparatus were to occur in the mesenteric smooth muscle cells, then the reduction in caffeine-induced vasoconstriction could be due to diminished contractile proteins rather than diminished Ca\(^{2+}\) release from the sarcoplasmic reticulum. Several lines of evidence indicate that reduced contractile protein expression cannot fully account for the reduced vasoconstrictor responses in mesenteric arteries. First, and most importantly, if alterations in the contractile apparatus were induced by unloading in the mesenteric arteries, then one would expect the myogenic vasoconstrictor response to be similarly affected, particularly at the highest pressures. The lack of a difference in the myogenic response within the physiological range of pressures (Fig. 2A) and the dependence of this constrictor response upon extracellular Ca\(^{2+}\) (12, 26, 33, 50, 51) indicate that the contractile apparatus in mesenteric smooth muscle cells from HU rats operates normally. Second, the reduction in mesenteric artery RyR2 expression would serve to reduce Ca\(^{2+}\) release from the sarcoplasmic reticulum, and this is consistent with the effects of unloading on portal vein myocytes (34). Thus, data from the present study indicate that the changes in mesenteric arterial vasoconstrictor properties occur, at least in part, through reductions in the RyR2-linked intracellular Ca\(^{2+}\) release mechanism within the vascular smooth muscle.

A diminished mesenteric arterial contraction could have several functional consequences. Because ~20% of the increase in peripheral vascular resistance during orthostasis normally occurs as a result of vasoconstriction in splanchnic organs (42), a diminished constrictor response within the splanchnic vasculature could potentially be an underlying mechanism of hindlimb unloading-induced orthostatic hypotension (55). Numerous studies indicate HU reduces constrictor properties of large conduit arteries (14, 16, 41, 43). In mesenteric arteries, the preponderance of evidence suggests HU diminishes contractile responses (5, 22, 38) (see Ref. 28 for exception). Results from the present study also demonstrate that the rapidity, in addition to the magnitude of vasoconstriction is diminished in HU rats. This is potentially significant in that delays in elevating total peripheral resistance could result in the destabilization of arterial pressure during the onset of orthostatic stress. This initial arterial hypotension, coupled with possible elevations in cerebral vascular resistance (1, 25), could serve to diminish cerebral perfusion and lead to syncpe. Thus, the results from the present investigation further support the notion that a diminished capacity of mesenteric resistance vessels to respond to vasoconstrictor stimuli and rapidly elevate peripheral resistance may contribute to post-flight and bedrest-induced orthostatic intolerance in humans.

A second functional consequence of the current findings could be a loss of aerobic capacity. Woodman et al. (57) observed that during treadmill exercise, HU rats had an impaired ability to reduce visceral blood flow to augment cardiac output, which is consistent with those observations of McDonald et al. (31). This relative inability to vasoconstrict splanchnic tissue is significant, since previous work has shown that ~10% of the blood flowing to active muscles at exercise intensities eliciting maximal oxygen consumption is the result of vasoconstriction in the splanchnic region (3). Together, these studies indicate that the diminished aerobic capacity associated with hindlimb unloading is due in part to an impaired ability to elevate visceral vascular resistance and thus redistribute blood flow and O\(_2\) to the active musculature.

The stimulus for the change in mesenteric artery vasoconstrictor responsiveness with hindlimb unloading is presently
unknown. Direct measures of blood flow (31) and the lack of change in vascular structure (22, 28, 56) indicate that neither blood flow nor arterial pressure are significantly altered when rats are in an unloaded position. It has recently been suggested that decrements in peripheral artery vasoconstrictor responsiveness may be associated with a circulating systemic factor(s) that is altered during unloading (5). Several circulating factors, such as atrial and brain natriuretic peptides, have been proposed (5) as mediators of the hyporesponsiveness to vasoconstrictor stimuli in the peripheral circulation.

Perspectives and Significance

The purpose of the present study was to determine whether unloading-induced deconditioning alters the intrinsic vasmotor properties of mesenteric resistance arteries, and, in particular, the rate of constriction, and to determine possible mechanisms for the reported decrements in mesenteric artery vasoconstriction. These data demonstrate that hindlimb unloading diminishes the vasoconstrictor responsiveness of mesenteric arteries to NE (Fig. 3A), KCl (Fig. 3B), and caffeine (Fig. 4) but does not affect myogenic vasoconstriction within a physiological range of pressures (Fig. 2A). The reduced vasoconstrictor response to caffeine along with the lower RyR2 mRNA and protein expression (Figs. 7 and 8) indicates that the attenuated vasoconstrictor responsiveness of mesenteric arteries is the result of an impaired intracellular Ca\(^{2+}\) release mechanism. If similar alterations in mesenteric resistance artery function occur in humans during prolonged exposure to microgravity or bedrest, this could be a contributing factor to orthostatic hypotension and reductions in aerobic capacity associated with cardiovascular deconditioning.

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and of sustained restraint on the intra-cerebroventricular pressure in rats. 


