Nonuniform changes in arteriolar myogenic tone within skeletal muscle following hindlimb unweighting

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Heaps, Cristine L., and Douglas K. Bowles. Nonuniform changes in arteriolar myogenic tone within skeletal muscle following hindlimb unweighting. J Appl Physiol 92: 1145–1151, 2002. First published October 26, 2001; 10.1152/japplphysiol.01031.2000.—Hindlimb unweighting (HLU) has been shown to alter myogenic tone distinctly in arterioles isolated from skeletal muscles composed predominantly of fast-twitch (white gastrocnemius) compared with slow-twitch (soleus) fibers. Based on these findings, we hypothesized that HLU would alter myogenic tone differently in arterioles isolated from distinct fiber-type regions within a single skeletal muscle. We further hypothesized that alterations in myogenic tone would be associated with alterations in voltage-gated Ca\(^{2+}\) channel current (VGCC) density of arteriolar smooth muscle. After 14 days of HLU or weight bearing (control), first-order arterioles were isolated from both fast-twitch and mixed fiber-type regions of the gastrocnemius muscle, cannulated, and pressurized at 90 cmH\(_2\)O. Mixed gastrocnemius arterioles of HLU rats demonstrated increased spontaneous tone [43 \(\pm\) 5\% (HLU) vs. 27 \(\pm\) 4\% (control) of possible constriction] and an approximately two-fold enhanced myogenic response when exposed to step changes in intraluminal pressure (10–130 cmH\(_2\)O) compared with control rats. In contrast, fast-twitch gastrocnemius arterioles of HLU rats demonstrated similar levels of spontaneous tone [6 \(\pm\) 2% (HLU) vs. 6 \(\pm\) 2% (control)] and myogenic reactivity to control rats. Neither KCl-induced contractile responses (10–50 mM KCl) nor VGCC density was significantly different between mixed gastrocnemius arterioles of HLU and control rats. These results suggest that HLU produces diverse adaptations in myogenic reactivity of arterioles isolated from different fiber-type regions of a single skeletal muscle. Furthermore, alterations in myogenic responses were not attributable to altered VGCC density.

HUMANS EXPOSED TO PROLONGED periods of weightlessness commonly exhibit adverse cardiovascular adaptations, including reduced plasma volume, orthostatic intolerance, and diminished aerobic capacity (5, 15, 26). The tail-suspended hindlimb unweighted (HLU) rat demonstrates cardiovascular adaptations similar to those observed in humans after prolonged periods of bed rest or microgravity (9, 10, 22, 27). Orthostatic hypotension, a potential contributor to orthostatic intolerance, may result from a diminished ability to sufficiently elevate peripheral vascular resistance within skeletal muscle to offset the significant reduction in plasma volume observed after prolonged exposure to microgravity (5). An inability to increase peripheral vascular resistance may result from an inadequate functional or structural adaptation of skeletal muscle arterioles to prolonged weightlessness.

Interestingly, previous studies have demonstrated that skeletal muscle arterioles adapt differently to HLU, depending on the muscle fiber type from which they are isolated. Specifically, arterioles isolated from skeletal muscles of predominantly slow-twitch fibers show no difference in spontaneous or myogenic tone or contractile responsiveness in HLU compared with control (CTL) rats but rather a structural remodeling resulting in a decreased arteriole diameter (7, 8). In contrast, arterioles of HLU rats isolated from muscle composed of primarily fast-twitch fibers demonstrate both functional and structural changes, i.e., reduced spontaneous and myogenic tone, decreased contractile responsiveness, and reduced arteriole wall thickness with no change in luminal diameter (7, 8). These findings suggest that adaptations in skeletal muscle arteriole responsiveness due to HLU may be partly attributed to the environmental milieu associated with the surrounding muscle fibers, as recently proposed (17). Therefore, we hypothesized that HLU would alter myogenic tone differently in arterioles isolated from distinct fiber-type regions within a single skeletal muscle.

Vessels displaying altered myogenic reactivity have also demonstrated altered KCl-induced contractile responsiveness (7). KCl-induced contraction is evoked by smooth muscle membrane depolarization and subsequent Ca\(^{2+}\) influx via voltage-gated Ca\(^{2+}\) channels. Furthermore, voltage-gated Ca\(^{2+}\) channel current (VGCC) is required for myogenic tone. Taken together, we further hypothesized that adaptations in VGCC density would contribute to altered myogenic reactivity.

Thus the purpose of this study was 1) to examine the effect of simulated weightlessness on myogenic tone of...
first-order arterioles isolated from both fast-twitch and mixed fiber-type regions of the gastrocnemius muscle and 2) to determine whether myogenic tone adaptations were associated with alterations in VGCC density of arteriolar smooth muscle.

METHODS

Animals. Male Sprague-Dawley rats (Harlan; 275–300 g body wt) were housed two animals per cage in a room with controlled temperature (24°C) and light (12:12-h light-dark cycle) conditions. Food and water were available ad libitum. The University of Missouri Animal Care and Use Committee approved all experimental procedures. After 7 days of acclimation to the animal facility, rats were assigned randomly to either a HLU or CTL group and housed individually. Rats assigned to the HLU group were acclimated to the unweighting procedure by having the hindlimbs temporarily suspended for 1–2 h/day for 3 consecutive days before initiation of the 14-day unweighting protocol. Hindlimbs of HLU rats were suspended with a harness attached to the tail as described previously (14, 20). Rats were suspended at an angle of 40–45°, which prevented the hindlimbs from touching any surface while allowing free, 360° movement about the cage on the forelimbs. CTL rats experienced normal weight-bearing activity during the 14-day treatment period. After completion of the 14-day HLU or CTL protocol, rats were anesthetized with pentobarbital sodium (50 mg/kg), and soleus and gastrocnemius muscles were removed. Soleus muscle weight was determined to evaluate efficacy of the HLU protocol (25).

Preparation of first-order arterioles. The gastrocnemius muscle was transferred to a dissection chamber containing cold (1–4°C) physiological saline solution (PSS) composed of (in mM) 143 NaCl, 2 CaCl₂, 1 MgCl₂, 5 KCl, 10 HEPES, and 10 glucose, pH 7.4. Under a dissecting microscope, feed arterioles leading to both the deep (mixed-fiber) and superficial (fast-fiber) gastrocnemius were identified. First-order arterioles in both muscles were defined as the first arteries within the epimysium that were supplied by the feed arteries and proximal to the first true bifurcation, as described previously (23). First-order arterioles from both the deep and superficial gastrocnemius were carefully dissected free of surrounding muscle tissue and transferred for cannulation to a Lucite vessel chamber containing cold PSS. The length of arteriolar segments isolated for cannulation was typically ~1 mm. Regions of both red and mixed muscle fiber types of the gastrocnemius surround the deep arteriole, whereas the superficial arteriole is surrounded by predominantly white fast-twitch muscle fibers (1). Arterioles were cannulated on one end with a glass micropipette filled with PSS; arterioles were tied securely to the pipette using 11-0 ophthalmic suture. The arteriole was gently flushed and the other end cannulated with a second micropipette and tied.

Microvessel video-dimensional instrumentation. The cannulated arteriole was transferred to the stage of an inverted microscope (Olympus IX50) equipped with a ×10 objective (numerical aperture of 0.25). The camera was coupled with a charge-coupled device video camera (Olympus 110), video monitor (Sony), and video micrometer (Microcirculation Research Institute, Texas A&M University, College Station, TX) for a final magnification of ×350 and a resolution of ~1 μm. Resolution of the system was calculated with the equation

\[ d = \frac{\lambda}{2NA} \]

where \( d \) is distance between two points, \( \lambda \) is visible light wavelength (400–700 nm), and NA is the numerical aperture of the objective. Data acquisition and analysis were accomplished with Axoscope 8.0 software (Axon Instruments, Foster City, CA). The micropipettes were connected to independent reservoir systems that were maintained at the same hydrostatic level to prevent flow through the vessel lumen. The height of the reservoirs was adjusted to set the intraluminal pressure of the arteriole at 90 cmH₂O. Leaks were detected by first pressurizing the arteriole to 90 cmH₂O and then verifying that intraluminal diameter remained constant when the valve to the reservoir system was closed. Only arterioles that were free of leaks were studied. The static vessel chamber bath (2 ml) was gradually warmed and maintained at 37°C for the duration of the experiment. Luminal diameter was continuously monitored throughout the experiment.

Experimental protocol. After a 1-h equilibration period at 90 cmH₂O, active myogenic curves were generated by slowly lowering intraluminal pressure to 10 cmH₂O and then increasing it from 10 to 130 cmH₂O by simultaneously raising both reservoirs in 20-cmH₂O increments. Active curves were generated in the presence of extracellular PSS containing 2 mM Ca²⁺. Intraluminal pressures were maintained for 4 min, which was sufficient for the vessel diameter to attain a steady-state response after the change in pressure. Concentration-response relationships for KCl were elicited at 90 cmH₂O by replacement of the extracellular bath with KCl solutions in which NaCl was replaced with increasing equimolar amounts of KCl. At completion of the experimental protocol, maximal (passive) intraluminal diameters of arterioles were measured at 20-cmH₂O increments from 10 to 130 cmH₂O intraluminal pressure by simultaneously raising both reservoirs in Ca²⁺-free PSS containing 1 mM EGTA and the Ca²⁺-channel blocker nifedipine (2 μM).

Smooth muscle cell dissociation. First-order arterioles from the mixed fiber region of the gastrocnemius were placed in low-Ca²⁺ (0.1 mM) physiological buffer containing 294 U/ml collagenase, 5 U/ml elastase, 2 mg/ml bovine serum albumin, 1 mg/ml soybean trypsin inhibitor, and 0.4 mg/ml DNase I. Cells were enzymatically dissociated by incubation in a 37°C water bath for 1 h. The enzyme solution was replaced with enzyme-free low-Ca²⁺ solution, and single smooth muscle cells were isolated by gentle trituration with a micropipette. Isolated smooth muscle cells were maintained in low-Ca²⁺ solution at 4°C until use (0–2 h).

Whole cell voltage clamp. Whole cell VGCC density was determined with the use of a standard whole cell voltage-clamp technique as used routinely (3, 4). Cells were initially superfused with low-Ca²⁺ (0.1 mM) PSS during gigaseal formation. After whole cell configuration, the superfuse was switched to PSS with tetraethylammonium chloride (75 mM) substituted isomolar for NaCl and 10 mM Ba²⁺ as the charge carrier. Heat-polished glass pipettes (2–5 MΩ) were filled with a solution containing (in mM) 120 CsCl, 10 tetraethylammonium chloride, 1 MgCl₂, 20 HEPES, 5 Na₂ATP, 0.5 Tris-GTP, and 10 EGTA, pH 7.1. Ionic currents were amplified by an Axopatch 200B patch-clamp amplifier (Axon Instruments). Whole cell currents were low-pass filtered with a cutoff frequency of 1,000 Hz, digitized at 2.5 kHz, and stored on a computer. Current densities (pA/pF) were obtained for each cell by normalization of whole cell current to cell capacitance to account for differences in cell membrane surface area. Capacity currents were measured for each cell during 10-ms pulses from a holding potential of ~80 mV to a test potential of ~75 mV. Capacity currents were filtered at a low-pass cut-off frequency of 5 kHz. Leak subtraction was not performed. Data acquisition and analysis were accomplished with the use of pCLAMP 8.0 software (Axon Instruments). Cells were continuously perfused under gravity flow. All experiments were conducted at room temperature (22–25°C).
Drugs and solutions. Reagents were obtained from Sigma Chemical with the exception of the ingredients for enzymatic dispersion, which were obtained from Worthington Biochemical. Stock solutions of nifedipine were dissolved in ethanol and diluted 1,000-fold for final solutions.

Data analysis. Spontaneous tone was calculated as \[1 - \left(D_0/D_f\right)\] × 100, where \(D_A\) and \(D_f\) are active and passive diameters, respectively. Myogenic curves are presented as actual vessel diameter (\(\mu m\)). To normalize for differences in initial and passive diameters between vessels, KCl constriction data are calculated as percent possible constriction: \[\left[D_B - D_{pass}/D_B\right] \times 100\], where \(D_B\) is the baseline diameter before intervention began and \(D_{pass}\) is the steady-state diameter measured at each concentration of KCl. Student’s unpaired t-tests were used to compare animal body mass, soleus mass, soleus-to-body-mass ratio, and spontaneous tone development between arterioles of HLU and CTL rats. Myogenic responses, concentration-response curves, and current-voltage relationships were compared with the use of two-way repeated-measures ANOVA with one within comparison and one between comparison, followed by post hoc tests for multiple comparisons when appropriate. Responses within arterioles of a single group were made using one-way repeated-measures ANOVA with one within comparison. Mean differences were ascertained using Bonferroni multiple comparison tests when a significant main effect was found with ANOVA. Experiments on vessels from CTL and HLU animals were performed on a paired basis; therefore, experimental order did not influence the results of this study. For all analyses, a \(P\) value of \(<0.05\) was considered significant. Data are presented as means ± SE, and \(n\) values reflect the number of animals, unless otherwise indicated.

RESULTS

Efficacy of HLU protocol. At the end of the 14-day unweighting protocol, body mass was significantly reduced 12% in HLU compared with CTL rats (314 ± 7 vs. 356 ± 10 g), as has been frequently reported in the literature (for review, see Ref. 25). Soleus muscle mass was 46% lower in HLU compared with CTL rats (98 ± 4 vs. 184 ± 14 mg, respectively). Importantly, soleus mass-to-body-mass ratio was 40% lower in HLU compared with CTL rats (0.31 ± 0.01 vs. 0.52 ± 0.04 mg/g, respectively), indicating a reduction in HLU soleus mass independent of differences in body mass. Soleus muscle atrophy confirms the efficacy of the HLU protocol (25).

Spontaneous tone and myogenic response. All first-order arterioles from the mixed-fiber gastrocnemius of HLU and CTL rats exhibited spontaneous tone, although HLU rats developed significantly greater tone than CTL rats at 90 cmH₂O intraluminal pressure (43 ± 5 vs. 27 ± 4%, respectively). This level of spontaneous tone in skeletal muscle arterioles from CTL rats is similar to that observed previously (7). Maximal (passive) intraluminal diameters of mixed gastrocnemius arterioles determined at 90 cmH₂O intraluminal pressure in Ca²⁺-free PSS were not significantly different between HLU and CTL rats (193 ± 12 and 231 ± 15 \(\mu m\), respectively), although arterioles from HLU rats tended to have a smaller passive diameter (\(P = 0.07\)). First-order arterioles from the fast-twitch gastrocnemius of HLU and CTL rats both exhibited similar levels of spontaneous tone (6 ± 2 and 6 ± 2%) and similar passive diameters (279 ± 31 and 315 ± 14 \(\mu m\)) at 90 cmH₂O intraluminal pressure.

Myogenic curves of both mixed and fast-twitch gastrocnemius arterioles of HLU and CTL rats are presented in Fig. 1. One-way ANOVA indicated that incremental elevations in intraluminal pressure increased active vessel diameter from 10 to 70 cmH₂O in mixed gastrocnemius arterioles from both HLU and CTL rats with no significant change in diameter from 70 to 130 cmH₂O (Fig. 1A). Two-way ANOVA demonstrated a significant treatment effect on active myogenic reactivity. Active diameters were significantly smaller in arterioles of HLU compared with CTL rats at all intraluminal pressures evaluated. Incremental elevations in intraluminal pressure increased passive vessel diameter from 10 to 130 cmH₂O in arterioles from both CTL and HLU rats (Fig. 1A). However, passive diameters were not different between arterioles of HLU and CTL rats at any pressure, as indi-

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**Fig. 1.** Effect of changes in intraluminal pressure on vessel diameter of first-order arterioles from control (CTL) and hindlimb-unweighted (HLU) rats. Pressure-diameter curves were generated under active and passive conditions for arterioles from both mixed (A) and fast-twitch (B) fiber-type regions of gastrocnemius muscle. A, active; P, passive; NS, not significant. Values are means ± SE; \(n\) (in parentheses) = no. of rats. *\(P < 0.05\) vs. CTL A.
cated by two-way ANOVA. In arterioles from the fast-twitch region of the gastrocnemius, incremental elevations in intraluminal pressure increased both active and passive vessel diameters from 10 to 130 cmH2O in both CTL and HLU rats (Fig. 1B). Furthermore, active and passive diameters were not different between arterioles of either HLU or CTL rats at any pressure (Fig. 1B).

**KCl concentration-response curves.** To examine potential mechanisms underlying the enhanced myogenic reactivity in mixed gastrocnemius arterioles of HLU rats, we evaluated concentration-response relationships for KCl. Examination of KCl curves provides information regarding voltage-dependent Ca2+ influx in intact vessels in addition to the electrophysiological determination of whole cell Ca2+ current density in isolated smooth muscle cells.

Increases in extracellular KCl produced concentration-dependent decreases in intraluminal diameter that were not significantly different in arterioles from HLU and CTL rats (Fig. 2). KCl-induced vasoconstriction in arterioles from the fast-twitch region of the gastrocnemius confirmed viability of these vessels (data not shown).

**Ca2+ channel current density.** The effect of HLU on whole cell Ca2+ current density in smooth muscle cells isolated from the mixed gastrocnemius arteriole was determined using 10 mM external Ba2+ as the charge carrier and current normalized to cell membrane capacitance (pA/pF). Cell capacitance (18.2 ± 1.4 vs. 20.1 ± 1.8 pF) was not different between smooth muscle cells isolated from HLU (n = 4 rats, 30 cells) and CTL (n = 4 rats, 22 cells) rats, respectively. Smooth muscle has been reported to contain both L-type and T-type Ca2+ channels are insensitive to this class of drugs (2). We evaluated the relative contribution of dihydropyridine-sensitive L-type Ca2+ channels to whole cell current density by using nifedipine (3 μM). Figure 3A shows representative current traces comparing VGCC in the absence and presence of nifedipine in cells from CTL and HLU animals. Peak and sustained inward currents were effectively abolished in the presence of nifedipine in cells from both CTL and HLU animals (peak: 96 ± 2 vs. 91 ± 2%, respectively; sustained: 101 ± 3 vs. 102 ± 3%, respectively), suggesting that L-type Ca2+ current is the dominant VGCC in smooth muscle cells from mixed gastrocnemius first-order arterioles and that the contribution of VGCC is not altered by HLU. Furthermore, current-voltage relationships for VGCC in smooth muscle cells isolated from arterioles of HLU and CTL rats are presented in Fig. 3B. No differences were seen in Ca2+ current density between cells of HLU and CTL rats, indicating that differences in VGCC activity were not responsible for changes in myogenic tone. Because we did not observe differences in spontaneous or myogenic tone between arterioles isolated from the white region of the gastrocnemius of CTL vs. HLU animals, we did not evaluate VGCC in these arterioles.

In addition to changes in current magnitude, Ca2+ influx via voltage-gated Ca2+ channels can be affected by shifts in channel activation parameters. For example, a negative shift in voltage-dependent channel activation would result in a greater VGCC activity and Ca2+ influx in response to a given membrane depolarization, independent of changes in maximal current. To test the possibility that voltage-dependent kinetics were altered by HLU, the voltage dependence of activation and steady-state inactivation for whole cell Ca2+ current were examined (Fig. 3, C and D, respectively). HLU had no effect on either voltage-dependent activation or inactivation. The membrane potential at which Ca2+ current decreased to one-half (inactivation) was not different in cells from HLU compared with CTL rats (Table 1). Furthermore, the membrane potential producing half-maximal activation was also not significantly different between smooth muscle cells of HLU and CTL rats (Table 1). Similarly, HLU had no effect on the slope components (slope) of activation or inactivation (Table 1).

**DISCUSSION**

We report that first-order arterioles surrounded by a mixed fiber-type region of the gastrocnemius muscle demonstrated increased spontaneous tone and enhanced active myogenic responses in HLU compared with CTL rats. In contrast, first-order arterioles surrounded by a fast-twitch fiber-type region of the gastrocnemius demonstrate similar levels of spontaneous tone and negligible myogenic responsiveness in HLU and CTL rats. These results are consistent with our hypothesis that HLU would alter myogenic tone differentially in arterioles isolated from distinct fiber-type regions within a single skeletal muscle. In contrast with our hypothesis, increased arteriolar smooth muscle...
VGCC density was not associated with enhanced myogenic tone observed in mixed gastrocnemius arterioles isolated from HLU rats.

With the use of the tail-suspended HLU rat model, previous studies have reported diverse functional and structural adaptations in skeletal muscle arterioles in response to HLU, depending on the muscle fiber type from which they are isolated (7, 8). These findings suggest that functional and structural adaptive responses of skeletal muscle arterioles to simulated weightlessness may be at least partly attributed to the physical and chemical environment associated with the surrounding muscle fibers (17). Our data support these findings and further document that diverse myogenic adaptations to HLU in arterioles isolated from distinct fiber-type regions can occur within a single skeletal muscle. Our data demonstrating no difference in myogenic responsiveness of white gastrocnemius first-order arterioles between CTL and HLU rats appear to conflict with previous findings of reduced myogenic tone in second-order arterioles isolated from the same muscle in HLU animals (7). Furthermore, myogenic reactivity of white gastrocnemius first-order arterioles from CTL animals was negligible compared with previous findings in second-order arterioles (7).

Table 1. Effect of hindlimb unweighting on voltage dependence of Ca\(^{2+}\) channel current

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<th>Activation</th>
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<th>Inactivation</th>
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<td></td>
<td>(K_{o.5})</td>
<td>Slope</td>
<td>(K_{o.5})</td>
<td>Slope</td>
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<tr>
<td>CTL</td>
<td>-1.4 ± 1.1</td>
<td>6.1 ± 1.0</td>
<td>-14.1 ± 1.5</td>
<td>-17.0 ± 1.5</td>
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<td>HLU</td>
<td>-0.8 ± 0.7</td>
<td>5.6 ± 0.7</td>
<td>-13.0 ± 1.8</td>
<td>-19.4 ± 2.0</td>
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Values are means ± SE; \(n\) values for control (CTL) and hindlimb-unweighted (HLU) rats are as indicated in Fig. 3.

Although fiber types in rat skeletal muscle are typically localized into discreet regions of uniform fiber type (distinct regions of fast- vs. slow-twitch muscle fibers) (1), human skeletal muscle is generally composed of a much more mixed fiber type, such that the proportion of slow- and fast-twitch fiber type is similar throughout all regions of the gastrocnemius muscle (11). Fiber types differ in the primary metabolic pathways utilized for energy production and thus produce different extracellular levels of metabolites, including...
lactate, H^+ , IMP, and osmolality during contractile activity. Therefore, the more mixed fiber-type composition of human skeletal muscle likely provides an environmental milieu different from that observed in rat skeletal muscle of uniform fiber type and may therefore produce adaptations in arteriole responsiveness different from those observed in arterioles of skeletal muscle composed of a more uniform fiber type. As such, the response of arterioles supplying skeletal muscle of a mixed fiber type to HLU may offer a more appropriate model of adaptations of human skeletal muscle arterioles to weightlessness.

We demonstrate that arterioles isolated from the mixed fiber-type region of the gastrocnemius respond to simulated microgravity with an increase in myogenic tone. Interestingly, we also observed a tendency for a reduction in the maximal, passive diameter in arterioles isolated from the mixed fiber-type region of gastrocnemius. As described previously, tissues exposed to chronic stimulation typically display immediate functional reactions followed by long-term structural adaptations (12). We postulate that the functional adaptive response of increased myogenic tone may be a temporary adaptation to reduce intraluminal diameter until a more permanent structural remodeling occurs to reduce cross-sectional diameter, an adaptive response similar to arterioles supplying the soleus muscle. The decrease in blood flow to the soleus is complete immediately after unweighting (18) and thus must be due to functional reductions in diameter of the resistance arteries supplying the soleus muscle. With more prolonged unweighting, arterioles feeding the soleus muscle remodel structurally to reduce diameter, and thereafter enhanced myogenic tone is not observed (7). The arterioles supplying muscle of mixed fiber type in the present study may be undergoing a similar, albeit slower, adaptive response. The tendency for a reduction in the maximal, passive diameter in arterioles isolated from the mixed fiber-type region of gastrocnemius may indicate that this arteriole continues to undergo changes to reduce intraluminal diameter; therefore, if hindlimbs were suspended for a more extended period of time, myogenic tone may return to control levels, as structural remodeling reduced passive diameter sufficiently to supersede the early adaptive response of increased myogenic tone.

The stimulus for this functional and potential structural adaptation may be associated with the environmental milieu associated with surrounding muscle fibers. Spatial patterns of muscle fiber-type recruitment with increasing levels of physical activity indicate that the red and mixed fibers surrounding the mixed fiber arteriole in our study are recruited under conditions of low-intensity activity, whereas the white fibers surrounding the superficial arteriole are recruited less frequently, only under conditions of high-intensity activity (1). These differences in recruitment patterns suggest that unweighting would more greatly influence the muscular activity and therefore the release of metabolites from fibers surrounding the mixed arteriole (17). Furthermore, the degree of muscle atrophy associated with different fiber-type regions may impact the arteriolar response to hindlimb unweighting. However, difficulties in physically delineating and isolating specific fiber-type regions of the gastrocnemius limited us in determining whether these specific regions demonstrated atrophy.

Previous studies examining the effect of HLU on myogenic reactivity of cerebral (13) and mesenteric (16) arterioles have postulated that changes in arteriolar pressure may contribute to the alterations observed in myogenic responses. Accordingly, one could speculate that the adaptive response of altered myogenic tone observed in these arterioles may occur to offset changes in arteriolar pressure. However, additional stimuli, independent of arteriolar pressure, are likely responsible for the altered myogenic responses observed. First, decreases in hindlimb arteriolar pressure due to hydrostatic changes occurring with suspension would likely be uniform throughout the hindlimb as opposed to the distinct myogenic adaptation found in arterioles from white vs. mixed gastrocnemius. Distinct adaptations of arterioles within the same muscle would require opposing changes in arteriolar pressure according to fiber type. Furthermore, the increased myogenic tone found in the arterioles from the mixed gastrocnemius, where intravascular pressure would be assumed to decrease, is similar to that observed in the cerebral circulation where increased intravascular pressure was postulated (13). The first-order arterioles examined in the present study are separated from aortic pressure primarily by the feed arteries. One possible mechanism for producing opposing changes in intraluminal pressure in mixed vs. white gastrocnemius arterioles would be opposing changes in vasoconstriction of feed arteries supplying mixed and white fiber arterioles. For example, dilation of the feed artery supplying the mixed gastrocnemius first-order arteriole would increase intraluminal pressure in this vessel, whereas conversely vasoconstriction of the feed artery supplying the white gastrocnemius first-order arteriole would decrease intraluminal pressure in this arteriole. Until intravascular pressure measures are obtained for the various vessels of interest, we cannot objectively evaluate transmural pressure as a primary stimulus for myogenic tone changes in our model.

Myogenic reactivity is highly dependent on Ca^{2+} entry via voltage-gated Ca^{2+} channels, as evidenced by a greatly diminished or complete loss of active myogenic tone in the presence of Ca^{2+} channel blockers or removal of extracellular Ca^{2+} (19). However, the myogenic response is almost certainly modulated by multiple cellular mechanisms. The finding that a change in VGCC density is not responsible for the enhanced myogenic tone observed in arterioles of HLU rats is contrary to our hypothesis and suggests that simulated weightlessness produces alterations in another mechanism(s) of the myogenic response, which remains to be determined. Previous studies have also demonstrated no effect of HLU on VGCC density in rat portal vein myocytes (21). Additional potential mechanisms of
myogenic reactivity that may have been altered by HLU in our study include alterations in other ion channel currents, such as Cl⁻ channel current or stretch-activated, nonselective cation channels, which would contribute to smooth muscle depolarization, increased Ca²⁺ sensitivity of the contractile machinery, and/or alterations in intracellular second messenger pathways. Interestingly, previous studies have demonstrated that complete blockade of dihydropyridine-sensitive VGCC only partially blocked stretch-induced Ca²⁺ entry, suggesting that stretch-activated Ca²⁺ entry may contribute to increased myogenic tone observed in arterioles of HLU rats in our study.

In conclusion, findings from the present study suggest that HLU increases myogenic tone in first-order arterioles isolated from a mixed fiber-type region of gastrocnemius skeletal muscle. In contrast, first-order arterioles supplying a fast-twitch fiber-type region of the gastrocnemius demonstrate similar myogenic reactivity in HLU and CTL rats. Furthermore, enhanced myogenic tone observed in mixed gastrocnemius arterioles isolated from HLU rats was not the consequence of enhanced VGCC density in smooth muscle cells isolated from these arterioles. We propose that the functional and structural adaptations demonstrated in arterioles isolated from a mixed fiber-type region of skeletal muscle may differ from those of arterioles isolated from muscles composed of either predominantly slow-twitch (soleus) or fast-twitch (white gastrocnemius) fibers due to changes in the environmental milieu associated with various muscle fiber-type regions after HLU. We further postulate that the functional adaptation of an increased myogenic tone in arterioles isolated from a mixed fiber-type region of the gastrocnemius may be a temporary adaptation to reduce intraluminal diameter until structural remodeling of the arteriole is complete.

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