Effects of biaxial stretch on arteriolar function in vitro

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Submitted 28 July 2006; accepted in final form 5 January 2007

Guo H, Humphrey JD, Davis MJ. Effects of biaxial stretch on arteriolar function in vitro. Am J Physiol Heart Circ Physiol 292: H2378–H2386, 2007. First published January 5, 2007; doi:10.1152/ajpheart.00810.2006.—Mounting evidence suggests that the normal biomechanical state of arteries may include a nearly equibiaxial intramural stress and that arteries tend to undergo rapid and dramatic remodeling when perturbed from this normal state. Technical developments since the early 1980s have enabled in vitro (acute) and ex vivo (chronic culture) study of isolated, perfused microvessels, and it is clear that these vessels share many functional similarities with arteries. To date, however, there has been no systematic study of the effects of in-plane biaxial loading on the biomechanical behavior of arterioles. Here we describe a modification to a prior in vitro arterial test system that allowed us to investigate the role of altered axial stretch on the passive, myogenic, and noradrenaline-stimulated biaxial behavior of isolated rat cremaster arterioles. We show that axial stretches from 85% to 110% of values often used in the laboratory and consistent with those normally experienced in situ induce modest changes in the measured mean circumferential and axial stress-strain behavior and in measures of distensibility and myogenic index. Nevertheless, altered axial stretch has a dramatic effect on the biaxial state of stress, and nearly equibiaxial stresses occur at axial stretches larger than those typically used in isolated arteriole studies. This finding is consistent with estimates of material and functional behavior in arterioles and suggests that long-term, ex vivo studies, wherein vessel growth and remodeling are critical, should be performed at higher axial lengths than have been used during most prior in vitro tests.

isolated arteriole; pressure-diameter; myogenic response; dose-response relationship; stress-strain

SMOOTH MUSCLE CONSTITUTES TWO-THIRDS of arteriolar wall volume (4, 6) and allows arterioles to actively constrict and relax in response to remote and local stimuli. Changes in arteriolar diameter are a primary determinant of local resistance and blood flow (11, 13), and arterioles are the major vascular segment controlling peripheral resistance. There is, therefore, a pressing need to understand well the biomechanics of arterioles.

The effects of smooth muscle activation on the wall mechanics of small arteries have been a topic of intensive investigation since the 1970s (9, 10). Improved methods for in vitro isolation, cannulation, and study of microvessels (15, 37) similarly enabled detailed functional studies, e.g., on the roles of smooth muscle activation in normal and diseased vessels (30, 31). Among local vasoactive control mechanisms, the pressure-induced myogenic response plays a primary role (7, 25), and isolated arteriole methods have been instrumental in elucidating cellular mechanisms underlying the myogenic response. Some of the associated mechanisms, such as the activation of voltage-gated channels and Ca2+ influx, are initiated by alterations in the mechanical state of vascular smooth muscle (34). Thus the myogenic response has been proposed to represent one type of mechanotransduction process (13) in vascular smooth muscle that may function to maintain wall tension and/or circumferential stress near a homeostatic value (11, 24, 28, 36).

Despite a number of important physiological and pharmacological studies on isolated, pressurized arterioles, the biomechanical properties of arterioles have not been characterized completely. For example, axial forces are known to significantly alter both the response of smooth muscle in (39) and the remodeling of (20) arteries; however, other than anecdotal reports, the influence of axial loading on arteriolar responsiveness has not been measured or quantified systematically.

The present study sought to characterize passive and active wall mechanics of arterioles under in-plane biaxial (circular) and axial) loading with reference to a well-defined, unloaded configuration. Such a study was motivated by the question of whether there is an “optimal mechanical state” for the myogenic response or agonist-induced vasoconstriction. We also expected that the high responsiveness of arteriolar smooth muscle would present new challenges for mathematical modeling; thus a second motivation was to collect experimental data sufficient for a nonlinear biomechanics approach to modeling arteriolar behavior similar to the approach used to study the mechanics of large arteries (18, 26).

METHODS

Preparation. All experiments were approved by the Animal Care and Use Committee of Texas A&M University. Male Sprague-Dawley rats (170–250 g, n = 22) were anesthetized with pentobarbital sodium (60 mg/kg ip). The right or left cremaster muscle was quickly exteriorized and excised, and the rat was killed by anesthetic overdose. The cremaster was placed in a cooled (4°C) dissection chamber filled with physiological salt solution (PSS) containing (in mM) 145 NaCl, 4.7 KCl, 2 CaCl2, 1.2 MgSO4, 1.2 NaH2PO4, 3 MOPS, 5 glucose, 2 pyruvate, and 0.02 EDTA as well as 1% bovine serum albumin (pH adjusted to 7.4 at 4°C). Segments of second-order arterioles (2A) were dissected carefully with sharpened instruments and transferred to a 3-ml chamber (12, 14). The bath chamber and cannulation pipettes contained PSS with the same ionic composition as the dissection buffer except that pH was adjusted to 7.4 at 37°C.

At room temperature, both ends of the arteriole were cannulated with micropipettes and secured with 11-0 ophthalmic...
suture (11, 30). The pipettes had tips pulled to ~50 μm internal diameter. The right pipette (stock = 1.97 mm OD, 1.79 mm ID; Drummond Scientific, Broomall, PA) was secured in a holder on a V-track microperfusion system (30), whereas the left pipette (stock = 1.5 mm OD, 1.0 mm ID; Garner Glass, Claremont, CA) was bent to a right angle using a microforge and then fit tightly into the tip of a compliant silicone tube. After the vessel was cannulated and the chamber transferred to the stage of a Zeiss inverted microscope (11), the pipette system was tested for pressure leaks, and a thin bronze wire connected to a force transducer (model 405A, 10 mN maximum force, Aurora Scientific, Aurora, ON, Canada) was hooked to the left pipette and affixed using a small drop of wax (Fig. 1).

The compliant tubing allowed the left pipette to move slightly to facilitate a consistent measurement of arteriolar axial force. The tubing-pipette assembly resembled a cantilever, which sustained two primary horizontal forces, $f_{\text{transducer}}$ and $f_{\text{arteriole}}$, in the leftward and rightward directions, respectively (Fig. 1, inset). Calibrations demonstrated that this setup provided a linear relation between the axial force imposed on the arteriole and that measured by the transducer ($f_{\text{transducer}} = 1.573 \times f_{\text{arteriole}}$, qualitatively consistent with a basic structural analysis; not shown), thus allowing simultaneous measurement of arteriolar axial force and intraluminal pressure in a functional arteriole.

During an experiment, the arteriole was pressurized using a static reservoir and continuously superfused at 0.4 ml/min with PSS using a roller pump. The microscopic image was displayed using a Dage MTI newvicon videocamera, and arteriolar inner and outer diameters were measured with a videomicrometer (11). Luminal pressure was monitored by a pressure transducer (Statham P23Db).

**Experimental protocols.** After initial pressurization to 58.5 mmHg (80 cmH2O) (35), the distance between the two pipette tips was increased by gradually moving the right pipette until the vessel was barely straightened. The internal diameter and arteriolar length were recorded using Zeiss ×16 and ×4 Achromat objectives, respectively. The initial axial length of the arteriole, referred to as the reference length, $L_P$, was measured as the distance between the two sutures that secured the vessel to the pipettes. Arterioles were then equilibrated in a heated bath (35—36°C) for at least 30 min until they developed spontaneous tone. Bath temperature was stable throughout the experiment so that it did not significantly influence the force measurements.

Vessels that developed spontaneous tone (a minimal 25% constriction in lumen diameter at 58.5 mmHg) were selected for testing either myogenic responses or norepinephrine (NE)-stimulated constrictions. Vessels were examined for myogenic responses by decreasing luminal pressure to 22.1 mmHg, then elevating pressure sequentially to 40.4, 58.5, 73.5, and 88.2 mmHg. At each prescribed pressure step and length, the inner diameter and the axial force were measured after 3 min of equilibration; hence, potential initial diameter and force transients were not recorded. A viable arteriole was tested at three to four different axial lengths between 85% and 110% of $L_P$. At a pressure of 58.5 mmHg, the length of the vessel was carefully adjusted, followed by 5—10 min of equilibration, after which the vessel was tested again for myogenic responsiveness at the new axial length. Each arteriole was usually tested first at its original length, then at a lower level of axial stretch, and, finally, at a higher axial stretch. Vessels selected for tests of NE responsiveness were maintained at an intraluminal pressure of 58.5 mmHg. With superfusion paused, the internal diameter and arteriole axial force were recorded 3 min after administration of NE to the vessel bath. NE was applied in cumulative doses, with the maximal dose being 10−6 M. Similar to tests of myogenic responses, NE responses were assessed at three to four different arteriolar axial lengths if the vessel remained viable. Between the tests at different levels of axial stretch, the bath solution was completely exchanged twice with fresh PSS.

After either of the above procedures, arterioles were allowed to equilibrate for at least 30 min in Ca2+-free PSS. Inner diameters and axial forces were then measured at pressures of 22.1, 40.4, 58.5, 73.5, and 88.2 mmHg. The vessels were equilibrated for 2 min at each prescribed pressure and length. Measurements were then taken under passive conditions for the same levels of axial stretch used to assess myogenic or NE responses. For each arteriole, wall volume was calculated at the different levels of axial stretch by recording both the inner and outer diameters at a pressure of 58.5 mmHg in Ca2+-free PSS.

![Fig. 1. Experimental setup of the arteriolar biaxial stretch test and corresponding free body diagram. A bronze wire connected to the transducer was hooked and secured by wax to the left pipette 5 mm below its connection to a compliant tube. The relative size of the arteriole was smaller than shown. $f_{\text{arteriole}}$ was the arteriolar axial force and the balance of force is illustrated in the free body diagram; $f_{\text{transducer}}$, axial force measured by transducer.](http://ajpheart.physiology.org/)
solution, and a mean wall volume was computed from the calculated volumes at each length.

A final step was to record the unloaded state of the arteriole. As the arteriolar lumen typically collapsed at zero intraluminal pressure, making measurement of inner diameter difficult and unreliable, intraluminal pressure was adjusted to 1.5–2.2 mmHg for a nearly unloaded state so that the arteriole was slightly distended to facilitate the recording of lumen diameter. After this, arteriolar axial length was slowly reduced to an axially unloaded state so that the shape of the arteriole became barely straight and the axial force ceased to decline perceptibly. Finally, the unique unloaded internal diameter \( D_0 \), unloaded axial length \( L_0 \), and unloaded axial force \( f_0 \), in which the latter represented the offset in the transducer, were recorded for each vessel.

Useful metrics. The circumferential stretch ratio \( \lambda_0 \) was used to delineate structural responses and was calculated as \( D/D_0 \), where \( D \) is the observed arteriolar internal diameter for a given intraluminal pressure.\(^1\) Mean circumferential (or hoop) stress was calculated as \( \sigma_0 = P\pi D / 2h \), where \( P \) is the intraluminal pressure and \( h \) is the deformed wall thickness. This formula provides the correct mean value of stress for both thin-walled and thick-walled vessels (note that, in the presence of residual stress, as measured in large arteries, the mean stress represents well the transmural stress distribution in a homeostatic state) \(^{(26)}\). Recall that the deformed thickness \( h \) was measured directly at \( P = 58.5 \text{ mmHg} \) in Ca\(^{2+}\)-free PSS solution and computed on the basis of the incompressibility of the wall at all other pressures. For comparison with data in the literature, an incremental circumferential stiffness (or modulus) was calculated as \( \Delta \sigma_0 / \Delta \lambda_0 \), where \( \Delta \sigma_0 \) is the change of circumferential stress resulting from a change in circumferential stretch, \( \Delta \lambda_0 \).

The axial stretch ratio \( \lambda_A \) was calculated as \( L/L_0 \), where \( L \) is the observed axial length. Mean axial stress was computed as \( \sigma_z = fL/A \), where \( f \) is the resultant axial force in the wall of the arteriole and the cross-sectional area of the wall, \( A \), is \( \pi h (D + h) \). Similar to the result for circumferential stress, this formula provides the correct mean axial stress regardless of wall thickness as long as cross-sectional area is computed exactly rather than via the thin-walled approximation (e.g., \( A = \pi h D \)). The force \( f \) was calculated as \( f = (f_{\text{arteriole}} + f_0) \), where \( f_{\text{arteriole}} \) is the axial force applied on the left pipette (Fig. 1, inset), which, as noted above, was calibrated to be \( (f_{\text{transducer}} - f_0) / 1.573 \); \( f_0 \) is an “extra” force arising from intraluminal pressurization and equals \( P\pi D^2/4 \). The stress ratio \( \sigma_0 / \sigma_z \) was used to characterize the biaxial state of stress experienced by the arteriole. In the absence of an applied axial force \( f_{\text{arteriole}} \), \( \sigma_0 / \sigma_z = 2(D + h)/D \) by mathematical deduction (which is \( \approx 2 \) for a thin-walled approximation), whereas it appears that \( \sigma_0 / \sigma_z = 1 \) in arterioles in vivo \(^{(26)}\), which emphasizes the importance of applied axial loads in vivo and by inference in tests in vitro or ex vivo.

Incremental distensibility \(^{(3)}\) and myogenic index \(^{(11, 38)}\) were calculated as \( 100(\Delta D / D) / \Delta P \) for passive responses and myogenic responses, respectively, where \( \Delta D / D \) is the fractional change in lumen diameter in response to each change in intraluminal pressure (\( \Delta P \)). The constriction ratio, defined by normalizing arteriolar diameter by the maximal diameter, was calculated as \( D/D_{P=22} \) for myogenic responses and \( D/D_{NE=9} \) for NE responses, where \( D_{P=22} \) is the arteriolar inner diameter at a pressure of 22.5 mmHg and \( D_{NE=9} \) is the inner diameter in response to NE of \( 10^{-9} \text{ M} \).

\textbf{In situ arteriolar length measurements.} To estimate the range of \( 2A \) lengths that would exist under physiological conditions in vivo, arteriolar lengths were measured in the intact cremaster muscle at \( \times 50 \) magnification using a Zeiss SV-8 dissection scope \((n = 4 \text{ rats})\). With the animal anesthetized, the left cremaster muscle was exteriorized with the testicle in place (i.e., the cremaster muscle was distended), and lengths of several \( 2A \)s in the central region of the muscle were measured using \( 3A \) side branches as reference points. The testicle was then removed through a small incision at the base of the cremaster muscle, the deferential artery connecting the testicle was ligated \((23)\), and a thin glass plate \((5 \text{ mm width } \times 5 \text{ cm long } \times 1 \text{ mm thick}) \) was inserted through the incision to support the central region of the muscle and permit length measurements of the same \( 2A \)s. The amount of muscle distention was the minimum possible that still allowed clear visualization of the arterioles, with \( 1A \)s and \( 2A \)s being distended axially just past the point of uncoiling. This configuration approximated the degree of distention of the muscle when the testicle was retracted into the abdomen.

\textbf{Data analysis.} For passive and NE responses, the axial stretch ratios were divided into three groups, i.e., low \((1.25 \leq \lambda_z \leq 1.55)\), moderate \((1.40 \leq \lambda_z \leq 1.50)\), and high \((1.55 \leq \lambda_z \leq 1.70)\) stretches. Only a few arteriolar axial stretch ratios fell below \(1.25\) or arose above \(1.70\), and stretch ratios that did not fit into the groups were excluded. For myogenic responses, low, moderate, and high stretch were defined similarly as \(1.22 \leq \lambda_z \leq 1.38\), \(1.40 \leq \lambda_z \leq 1.50\), and \(1.53 \leq \lambda_z \leq 1.70\), respectively, because of a smaller number of samples. The averages for the axial stretch ratios were similar for both groups, however (Table 1).

Results were tested for statistical significance using one-way ANOVA followed by post hoc analysis (Tukey test) for multiple comparisons. Differences were considered significant at the \( P < 0.05 \) level.

\textbf{RESULTS}

\textbf{Morphological data.} Arterioles were similar in size, independent of grouping, in both unloaded and loaded configurations (Table 2). That is, unloaded lengths and diameters were not significantly different, and arteriolar diameters and wall thicknesses were comparable at a pressure of 58.5 mmHg.

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
& Low Stretch & Moderate Stretch & High Stretch \\
\hline
Passive & \( 1.31 \pm 0.01 \) (10, 8) & \( 1.45 \pm 0.01 \) (11, 11) & \( 1.61 \pm 0.02 \) (12, 11) \\
Myogenic & \( 1.30 \pm 0.02 \) (8, 6) & \( 1.45 \pm 0.02 \) (6, 6) & \( 1.61 \pm 0.03 \) (7, 5) \\
NE & \( 1.31 \pm 0.01 \) (7, 6) & \( 1.45 \pm 0.01 \) (14, 12) & \( 1.60 \pm 0.02 \) (12, 8) \\
\hline
\end{tabular}
\caption{Axial stretch ratios for the three experimental groups}
\end{table}

\footnotesize{\(^{1}\) Note that \( \lambda_A \) (a component of the left stretch tensor) is preferred, in general, over components of the often-used linearized strain tensor (\( \epsilon_0 = \lambda_0 - 1 \)), for the former is exact and independent of rigid body motion, whereas the latter is approximate and sensitive to rigid body motions \((26)\).}
Table 2. Morphological characteristics of the arterioles in unloaded and pressurized conditions in Ca\textsuperscript{2+}-free PSS

<table>
<thead>
<tr>
<th>Group</th>
<th>Passive</th>
<th>Myogenic</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>(L_0), (\mu)m</td>
<td>661.4±43.3</td>
<td>647.0±52.3</td>
<td>611.5±55.8</td>
</tr>
<tr>
<td>(D_0), (\mu)m</td>
<td>66.0±4.2</td>
<td>56.8±3.2</td>
<td>59.6±3.6</td>
</tr>
<tr>
<td>(D_f), (\mu)m</td>
<td>130.3±5.7</td>
<td>126.5±1.5</td>
<td>126.8±4.4</td>
</tr>
<tr>
<td>(h_0), (\mu)m</td>
<td>9.8±0.7</td>
<td>11.0±1.1</td>
<td>9.9±0.8</td>
</tr>
<tr>
<td>(\lambda_0)</td>
<td>1.47±0.02</td>
<td>1.46±0.02</td>
<td>1.46±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), number of arterioles tested for each response. Only one vessel was tested for each animal. PSS, physiological salt solution; \(L_0\) and \(D_0\), arteriolar unloaded length and unloaded diameter, respectively; \(D_f\) and \(h_0\), arteriolar inner diameter and wall thickness at the initial axial stretch and intraluminal pressure of 58.5 mmHg, respectively; \(\lambda_0\), original axial stretch ratio at which the vessel was tested.

As illustrated in Fig. 3, the ranges of circumferential stretch ratios were different between passive and active responses. As lumen diameters were reduced during active responses to pressure and NE, the circumferential stretch ratios were accordingly lower. Moderate and high axial stretch slightly flattened the myogenic stress-strain curve (Fig. 3B) relative to the low-stretch case, unlike passive responses in which the shape of the stress-strain curve was almost unaffected (Fig. 3A). Again, there was a statistically significant effect of high axial stretch under axial stretch. On the basis of statistical tests, arterioles were stretched nearly to the same degree (\(\lambda_0\)) when the protocols were started. In situ measurements of 2As indicated that there was a 21.1 ± 3.1% increase in 2A length (\(n = 10\) vessels) when the cremaster muscle was distended by the testicle, compared with when the testicle was retracted.

**Diameter versus pressure or NE.** Relationships between diameter and either intraluminal pressure or concentration of NE at a fixed pressure reflect the overall structural stiffness of a vessel; they are shown in Fig. 2. Vessels at low-, moderate-, and high-stretch levels all exhibited nonlinear stiffening passive responses (Fig. 2A), developed myogenic responses (Fig. 2B), and constricted to NE (Fig. 2C). As expected, one effect of the high axial stretch was a smaller diameter at each pressure. For the ranges studied, however, the effect of high axial stretch was statistically significant only for the myogenic response group at pressures ≥58.5 mmHg.

**Mean circumferential stress versus circumferential stretch ratio.** Relationships between circumferential stress and circumferential stretch reflect the intrinsic material properties of the wall (Fig. 3). For passive responses, high axial stretch decreased the arteriolar luminal diameter and accordingly lowered the circumferential stretch ratio, which led to a leftward shift of the circumferential stress-stretch curve while preserving the shape of the curve (Fig. 3A); this change was not statistically significant, however. Not surprisingly, the associated incremental stiffnesses at different axial stretch ratios were also not significantly different from each other (e.g., ~540 kPa at a moderate stretch and an intraluminal pressure of 58.5 mmHg).

Circumferential stress-stretch curves for passive and active responses were very different in character. The passive response was highly nonlinear, similar to that of arteries, whereas relationships for the myogenic and NE groups were substantially more linear. Because the increase in pressure during myogenic responses numerically played a greater role in the calculation of circumferential stress than did the consequential decrease in lumen diameter, this stress increased with intraluminal pressure even though lumen diameter decreased (Fig. 3B). When compared with passive responses, arteriolar lumen diameters during myogenic responses were much smaller, whereas wall thicknesses were much greater at the equivalent intraluminal pressures. Therefore, mean circumferential stresses were smaller, based on the law of Laplace.
only in the myogenic response group at pressures \( \geq 58.5 \) mmHg.

Stress-stretch curves for NE responses were measured while intraluminal pressure was kept constant at 58.5 mmHg. It is interesting that the stress-stretch relationships at low-, moderate-, and high-stretch levels all appeared nearly linear (actually mildly quadratic as expected theoretically) and were very similar to each other (Fig. 3C). The stress ranges for the NE groups were similar to those during myogenic responses. When compared with passive responses, arterioles during both myogenic and NE responses maintained their stress at a much lower level, i.e., around 20 to 60 kPa.

Mean axial stress versus axial stretch ratio. As data for three different groups of axial stretch ratios (Table 1) might not adequately illustrate the characteristics of the axial stress-stretch curves, test results at all axial stretch ratios were included and categorized into four groups (Fig. 4). Increases in luminal pressure led to an upward shift of the axial stress-stretch curve for passive responses (Fig. 4A), which demonstrated the effect of the “extra force,” \( f_p \), denoted in Fig. 1. Owing to the diminished effect of \( f_p \) during vasoconstriction, the axial stress curve shifted downward for myogenic responses while intraluminal pressure increased (Fig. 4B). When intraluminal pressure was low, axial stresses during myogenic responses were comparable with those during passive responses. When intraluminal pressure was elevated, however, axial stress was much lower if arterioles were regulated by myogenic constriction.

Finally, the axial stress-stretch curves during NE stimulation (Fig. 4C) were similar to those for the passive responses at a...
pressure of 58.5 mmHg (Fig. 4A), which was the intraluminal pressure used for all tests of NE responses. These results are consistent with the predominant direction of smooth muscle force being in the circumferential direction (compare Fig. 3, A and C) (41).

**Stress ratio versus pressure and NE.** The stress ratio \( \sigma_x/\sigma_z \) represents the in-plane, biaxial state of stress in the arteriolar wall. Since this metric was calculated as the ratio of circumferential stress to axial stress, the stress ratio curves shifted downward when the axial stretch ratio increased (Fig. 5). Stress ratios for both passive and myogenic responses increased slowly with elevation of intraluminal pressure at low and moderate axial stretches (Fig. 5, A and B) but changed little with pressurization at high axial stretch. On the other hand, stress ratios decreased slightly at low and moderate axial stretches in response to higher doses of NE (Fig. 5C) but again changed little at high axial stretch. Recall that this ratio equals 2 by the thin-wall approximation when there is no applied axial force; values above 2 thus reveal that \( \sigma_x/\sigma_z \) was negative, suggesting that the vessel was bent slightly or that bending was imminent as expected of a vessel that is not stretched sufficiently in the axial direction. Conversely, values below 2 reveal that \( \sigma_x/\sigma_z \) was positive, with values close to 1 thought to correspond to the homeostatic state in large arteries (26). It is interesting to note, therefore, that the stress ratio was close to 1 in the moderate- and high-stretch myogenic tests at pressures between 40.4 and 58.5 mmHg, close to that expected at the in vivo pressure (35).

**Incremental distensibility, myogenic index, and constriction ratio.** Incremental distensibility, determined by the slope of the pressure-diameter relationship, as \( 100(\Delta D/\Delta P) \), describes the structural behavior of arterioles in the passive condition. As the pressure-diameter curves at multiple axial stretches largely paralleled and overlapped each other (Fig. 2A), the curves of incremental distensibility likewise overlapped (Fig. 6A). Note, too, the near constancy of distensibility at \( P \geq 58.5 \) mmHg.

The myogenic index is given by the same formula and represents the change in diameter per change in intraluminal pressure (21). As the arterioles showed consistent constriction in response to increased luminal pressure within our test range, the myogenic index was always negative. Note, too, that between \( \sim 40 \) and 75 mmHg, the gradual decrease in myogenic constriction reflected the characteristics of the curves in Fig. 2B.

The constriction ratio was used to characterize and compare the level of active responsiveness, i.e., myogenic constriction (Fig. 7A) or NE constriction (Fig. 7B), by normalizing each...
Responses and values are means \( \pm \) SE. No significant difference was found \( P < 0.05 \).

Diameter to the baseline diameter (i.e., \( D_{P-22} \) for myogenic responses and \( D_{NE-0} \) for NE responses). As seen from Fig. 7, the constriction ratios decreased monotonically as a function of increased intraluminal pressure or concentration of NE. Although differences due to altered axial stretch were not significant, there was a trend toward a greater myogenic response at the highest level of axial stretch. Statistically, however, an optimal axial stretch for smooth muscle contractility was not obvious over the range studied. Finally, the constriction ratio during stimulation by NE was nearly linear in terms of concentration and greater in magnitude than that induced by pressure.

**DISCUSSION**

Quantifying the biomechanical properties of arterioles is essential for understanding both their role in local control of blood pressure and flow and their adaptation to altered mechanical stimuli. It is now clear that the normal biomechanical state experienced by a blood vessel must be known to understand fully how altered hemodynamic loads perturb the vessel from its normal state. Previous studies on the physiology and mechanics of arterioles have focused primarily on the circumferential properties of these vessels, usually in terms of circumferential stress \((4, 7)\), wall tension \((1, 24)\), distensibility \((3, 5)\), or growth index \((32)\). Recent findings on arteries suggest, however, that axial loads may be equally as important as circumferential loads in defining the normal biomechanical state (e.g., stress and stretch). It appears, for example, that the normal in-plane state of stress in the arterial wall may be nearly equibiaxial, with values around \( \sigma_{P} \approx \sigma_{Z} \approx 100 \text{ kPa} \) \((27)\). When perturbed from their normal axial extension, arteries appear to remodel quickly to restore the axial stretch or stress toward baseline \((8, 19, 22, 29)\). A goal of the present study, therefore, was to explore the effects of a range of in-plane biaxial loads (i.e., axial as well as circumferential) on the passive and active behavior of arterioles under well-controlled conditions in vitro.

With the use of methods of microvessel cannulation and perfusion \((14–16)\), arterioles are typically stretched axially to a reference length determined empirically, or approximating an in vivo length, and then subjected to functional or mechanical protocols \((2, 11, 24, 32, 42)\). That is, there has been no rigorous standard for setting the axial length. A few studies have addressed the role of axial stretch on arteriolar reactivity, but only in an anecdotal manner. For example, it was reported that a roughly 25% increase over the “normal” testing length did not affect constrictions to pressure or to NE in isolated rat cremaster arterioles \((24)\). However, in the same study, an acute increase in intracellular \( \text{Ca}^{2+} \) concentration was observed when axial stretch was applied and the \( \text{Ca}^{2+} \) signal declined to a level close to baseline within 3 min. In another study of isolated rabbit coronary microvessels \((17)\), it was reported that an “extra 40% stretch” increased myogenic tone. Axial stretch has also been reported to alter the contraction force of bovine lymphatic vessels \((33)\). In the latter two cases, no quantitative details were given as to the effect of axial length on the magnitude of the respective responses. Furthermore, in none of the above studies was axial force recorded nor was any attempt made to define axial stretch relative to a unique reference. Our experiments address both of these gaps. On the basis of the present study, we recommend that a true unloaded axial length \( L_0 \) should be recorded. In this way, the overall axial stretch ratio (i.e., extension) can be written as \( \lambda_z = \lambda_z^* \Lambda_z \), where \( \lambda_z^* \) is the ratio of the current length \( L \) to the commonly adopted test length \((e.g., L_p=so)\) and \( \Lambda_z \) is the ratio of the commonly adopted length to the true unloaded length \( L_0 \). This would facilitate the comparison of results between laboratories, species, and vascular beds.

Arteriolar axial stretch levels between 85% and 110% of the original test length \( L_p \) were selected because we found in pilot tests that stretch to <85% of the original length would always cause bending at intraluminal pressures >73 mmHg. Conversely, when the axial stretch of cremaster arterioles was >110% of the original value, axial force increased markedly and the arteriole underwent overt deformation. We were also concerned that greater stretch would lead to irreversible damage. Thus we decided not to stretch the arterioles more than 110% of \( L_p \). Similarly, the range of intraluminal pressures and doses of NE were chosen carefully to avoid higher pressures (e.g., >88 mmHg) and NE concentrations (e.g., >10^{-6} M) that might cause irreversible effects.

Figures 3A and 4A reveal that the passive biaxial behavior of arterioles is very similar to that of arteries \((26)\). Both the mean circumferential and the mean axial stress-stretch relationships are highly nonlinear, and differences between the circumferential and axial curves reveal a strong anisotropy (e.g., the axial response is more bilinear with lower stiffness at lower extensions). Moreover, increasing the axial stretch leads to a decrease in the circumferential distensibility, whereas increasing pressure has less of an effect on the axial behavior.
Interestingly, at a pressure of ~60 mmHg and an axial stretch of ~60%, relative to the unloaded configuration, the passive biaxial state of stress is ~100 kPa, similar to that previously determined for arteries (27). It should be noted that this strong similarity with arteries was manifested despite a basic difference in testing procedure. Whereas passive arteries are typically tested under cyclic loading to reduce hysteresis (18), the tests herein were performed via incremental loading. Our quasistatic incremental loading strategy without preconditioning allowed for short-term stress relaxation before data collection. In both classes of tests, therefore, viscoelastic effects are minimized.

Similar to the anisotropic passive behavior, Figs. 3C and 4C reveal a strongly anisotropic behavior when the vessels were contracted with NE. The linear circumferential response over the range of stretches considered is in stark contrast to the nearly bilinear axial response. In particular, similar to the passive response, an axial stretch ~50% appeared to be the point at which the vessel stiffened markedly, likely due to straightening of axially oriented adventitial collagen. We note that the in vivo state of many soft tissues tends to be near such “elbows” in the stress-stretch response, an issue that we revisit below.

Figures 6 and 7 suggest that myogenic responsiveness was not altered significantly by the range of axial stretches applied in our study. We considered two different metrics of myogenic reactivity: the myogenic index (Fig. 6), which has been commonly used to characterize and quantitate myogenic vasoconstriction (21), and a constriction ratio, which represents the constrictive characteristics of arteriolar lumen and facilitates comparisons between pressure-induced (myogenic) and agonist-induced (NE) vasoconstriction (40). In both cases, there were no significant differences between the responses of vessels at the four levels of axial stretch between 0.85 and 1.1 of our reference value. These results are in apparent contradiction to anecdotal reports in the literature that axial stretch enhances arteriolar (17) or lymphatic (33) reactivity. It could be argued that we failed to test a sufficient range of axial lengths in our protocols, but we used a range of axial loads widely described in the literature and one that approximated the same range of axial length changes as would occur under physiological conditions in vivo. Axial lengths below 0.85 of \( L_p \) led to buckling of the vessels at high intraluminal pressures and made inner-diameter measurements difficult and inaccurate. Axial lengths over 1.1 \( L_p \) led to overt arteriolar deformation and an abrupt increase in axial force that approached the upper limit of the force transducer range. It would have been possible to stretch the vessels axially to lengths that would have produced obvious damage, but such procedures are unlikely to be used by experienced investigators wanting to preserve normal vascular reactivity. Another possibility for the apparent discrepancy may be the results and others (17, 33) is that previous authors may have been referring to transient responses (e.g., pressure-induced constrictions) that were not maintained at the 3-min measurement point used in our study. Alternatively, those reports may have been referring to the speed at which the vasomotor responses occurred. Neither of these parameters was measured in our study. It is also possible that arterioles from other vascular beds and lymphatics are much more distensible axially than the cremaster muscle arterioles used in our study.

Like arteries (26), the achieved circumferential and axial stresses both appear to be ~30 to 70 kPa at pressures of ~60 mmHg and axial stretches ~60% relative to the unloaded reference. Unlike arteries, the operative range of circumferential stress differs dramatically between the passive (Fig. 3A) and active (Fig. 3, B and C) behaviors. Likewise, the values of circumferential stress are much less in the active state. Both of these differences are due, in large part, to the strong constriction by arterioles. Our in vitro results thus likely provide bounds for the behavior expected in vivo when basal vascular tone is present. We find that changes in axial stretch from 85% to 110% of a commonly used reference for arteriolar tests (from ~31% to 60% stretch relative to an unloaded reference) induce modest changes in the circumferential and axial stress-stretch responses (Figs. 3 and 4) and little change in overall distensibility and myogenic index (Fig. 6). The relative insensitivity of constrictor responses to axial stretch could be a mechanism to preserve vascular reactivity over the range of axial loads experienced under physiological conditions. Nevertheless, as expected, changes in axial stretch cause significant changes in the biaxial state of stress at a given pressure. In particular, Fig. 5 shows that the ratio of circumferential to axial stress tends to vary little as a function of pressure and contractility when the arterioles are maintained at the high-stretch level. We conclude, therefore, that if arteries do prefer a nearly equibiaxial state of stress (i.e., if the intramural cells modify wall geometry, structure, and properties when perturbed from such a state of stress) and if arterioles have similar mechano-biological controls, then accurate measurement and control of axial stretch in biomechanical tests on arterioles are required. In particular, our results suggest that axial extensions used in prior studies based on experience (e.g., the lack of bending of the vessel when pressurized to a target value) may well have been below the preferred value. Although our results show that this may not have markedly affected acute responses to pressure or NE, they suggest that much more care needs to be given to the degree of axial extension used in many in vivo studies (e.g., cheek pouch and cremaster muscle preparations) and ex vivo (e.g., perfused and cultured vessel models), particularly to ensure that the stress ratio approaches a value of unity. Moreover, because mechanobiological cell-matrix and cell-cell interactions are governed largely by conformational changes to membrane surface receptors (integrins and cadherins), which, in turn, would be affected by the biaxial state of stress, there is a need to include such considerations in future studies.

ACKNOWLEDGMENTS

The authors are grateful to Judy Davidson and Andy Piwonka for technical assistance and to Dr. Rudolph Gleason for helpful suggestions.

REFERENCES


AJP-Heart Circ Physiol • VOL 292 • MAY 2007 • www.ajpheart.org