Force-velocity relationship of myogenically active arterioles

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Davis, Michael J., and Judy Davidson. Force-velocity relationship of myogenically active arterioles. Am J Physiol Heart Circ Physiol 282: H165–H174, 2002.—We compared the shortening velocity of smooth muscle in arterioles that had low or high levels of myogenic tone or norepinephrine (NE)-induced tone. We hypothesized that enhanced myogenic tone of arterioles reflects an enhanced maximum velocity of shortening of arteriolar smooth muscle in a way that is different from that produced by NE. These concepts are untested assumptions of arteriolar mechanics. Second-order arterioles from hamster cheek pouch (passive diameter at 40 mmHg = 42 μm) were isolated and cannulated for in vitro study. In the absence of flow, pressure was controlled by hydraulic pumps so that servo control of wall tension could be achieved from measurement of internal diameter and pressure. Isotonic quick-release protocols were used to measure the initial velocity of shortening following release from control wall tension (afterload) to a series of fractional afterloads. After release, the initial rates of shortening were fit to the Hill equation to obtain coefficients for a hyperbolic fit of the velocity-afterload relationship. The maximal unloaded shortening velocity for partially activated arterioles ($V_{max}$) was determined from the y-intercept of each plot. Using this procedure, we compared $V_{max}$ from two groups of arterioles equilibrated at low or high pressure, i.e., with low or high myogenic tone. Arterioles with higher myogenic tone had higher velocities than arterioles with lower myogenic tone. $V_{max}$ for arterioles partially activated with NE at low pressure was comparable to $V_{max}$ for arterioles with high myogenic tone, but NE produced high velocities at low force, whereas enhanced myogenic tone produced roughly parallel shifts in velocity and force. The results suggest that increased myogenic tone does indeed reflect enhanced activation of arteriolar smooth muscle, and this effect is mechanically different from that produced by NE.

maximal shortening velocity; arteriolar mechanics; isolated arterioles; norepinephrine; agonist-induced tone

THE MYOGENIC RESPONSE is an inherent ability of smooth muscle to respond to increased force. In arterial blood vessels, the myogenic response is manifested as a sustained constriction following luminal pressure elevation. The time course of a vascular myogenic constriction occurs in several phases. In most vessels, a rapid elevation in luminal pressure produces an immediate distention. The amount of distension is variable and depends on the level of initial tone (10). After the distension phase, a contraction begins to develop. In large vessels, the delay before vascular smooth muscle (VSM) contraction varies from many seconds in arteries to a fraction of a second in arterioles (17). The amount of constriction is also greater in arterioles (3, 24). In some vessels, the constriction is biphasic, with an exaggerated initial phase that is independent of the rate of pressure rise, followed by a partial relaxation to a steady-state phase (9, 10). The steady-state constriction appears to be maintained almost indefinitely (18) and accounts for the basal tone of resistance vessels in almost every organ.

Implicit in the concept of a myogenic constriction is the assumption that VSM shifts to a higher activation state. Johnson (16) stated this assumption clearly in his landmark chapter in the Handbook of Physiology, basing his conclusion on length-tension analyses of in vivo arterioles from cat mesentery (15). Specifically, enhanced activation associated with myogenic tone was predicted to explain the progressive, leftward shift of calculated arteriolar wall tension with pressure. Whereas Johnson's initial analyses required arteriolar pressures to be extrapolated from measurements of systemic arterial pressure, direct measurements of arteriolar pressure in subsequent experiments confirmed Johnson's predictions (6).

However, analyses of the myogenic response in vivo are subject to concerns about modulation by parenchymal cell metabolites and endothelial cell vasoactive factors because changes in oxygen delivery and flow necessarily accompany changes in perfusion pressure (8). Thus it is important that these protocols be repeated in isolated arterioles where the endothelium is denuded and/or where changes in flow are prevented. Even so, such evidence would only qualitatively support the concept of an increased VSM activation state. A more rigorous test of this idea would be to demonstrate that increased myogenic tone reflects an increase in maximal velocity of shortening ($V_{max}$) of arteriolar smooth muscle. However, measurements of VSM force-velocity relationships have only been possi-
ble, to date, on larger blood vessels that lack significant myogenic responsiveness (1, 11, 14, 20, 21).

In this study, we tested the hypothesis that myogenic activation of arterioles is associated with an increase in unloaded shortening velocity of arteriolar smooth muscle. To make these measurements, we performed isotonic release protocols on isolated, cannulated segments of second-order (2A) hamster cheek pouch arterioles using a servo-control system to regulate wall tension. These vessels have been studied extensively in vitro and generate ~50% myogenic tone (relative to maximal passive diameter), which is comparable to 2A tone observed in vivo. Furthermore, maximal length-tension relationships for these vessels have been previously determined (4). As an additional aspect of this study, we compared how enhanced myogenic tone and agonist-induced tone might differentially alter the force-velocity relationship of arteriolar smooth muscle.

METHODS

**Isolated arteriole preparation.** Golden Syrian hamsters (120–180 g) were anesthetized with intraperitoneal injections of pentobarbital sodium (60 mg/kg). One cheek pouch from each animal was cleaned, excised, and placed in 4°C physiological saline solution containing 1 g/100 ml albumin. All animal protocols conformed to institutional guidelines.

To perform isotonic release protocols, several substantial modifications were made to our standard isolated arteriole measurement system. To maximize the frequency response of the system and reduce the possibility of cannulation-induced trauma, vessels were studied in a parent-daughter arrangement as previously described (3, 10). After pinning was completed, the avascular connective tissue covering the pouch was removed, and arteriolar segments consisting of a first-order (1A) parent vessel and 2A daughter vessel were selected for study. The segments were carefully cleared of connective tissue with the use of sharpened instruments, excised, and transferred in a Pasteur pipette to a temperature-controlled chamber for cannulation and study (7). At room temperature, both ends of the 1A were cannulated with ~60-μm (external diameter) micropipettes and secured with 12-0 suture. Cannulation pipettes contained the following solution (in mM): 145.0 NaCl, 5.0 KCl, 2.0 CaCl₂, 1.2 MgSO₄, 1.2 NaH₂PO₄, 0.02 EDTA, 5.0 glucose, 2.0 pyruvate, 2.0 3-(N-morpholino)propanesulfonic acid, and 1 g/100 ml purified bovine serum albumin. The 1A segment was intentionally overstretched in the longitudinal direction to eliminate tone and possible interference with myogenic responses of the daughter vessel through conducted pressure responses (22). The second-order (daughter) side branch (segment length ~ 400–700 μm) was tied off at its distal end with 12-0 suture and stretched to approximately its in vivo length (measured before dissection) using a suction micropipette.

After the chamber and pipette system were transferred to the stage of an inverted microscope, the temperature was slowly raised over 1 h to 36–37°C by a circulating water bath. A roller pump (Gilson Milipuls 3) was used to superfuse (4 ml/min) the vessel segment with a Ringer-bicarbonate solution (RBS) containing 131.9 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, and 18.0 NaHCO₃ and bubbled continuously with 95% N₂-5% CO₂ (pH = 7.35; PO₂ ~50 mmHg). During this time, one end of the vessel segment was pressurized from a reservoir to its normal in vivo pressure while luminal pressure was monitored at the other end using a pressure transducer. Differences between input and output pressures were indicative of leaky vessel segments, and data from leaky segments were not used for further analysis. The amount of myogenic tone was referenced to the passive diameter at 40 mmHg (Lp), which is close the normal in vivo pressure of 2As (4). This value was always measured at 22°C during the initial equilibration period before the vessel gained tone, but in several vessels a complete passive pressure-diameter relationship was obtained at the end of the experiment after equilibration in Ca²⁺-free RBS containing 10⁻⁴ M nitroprusside.

**Microscope system.** Each vessel segment was transilluminated using a Zeiss IM-35 inverted microscope with a heat-filtered, 100-W tungsten light source, filtered at 515 nm. The 2A branch was visualized at high magnification using a Leitz ×75 oil immersion objective (numerical aperture = 1.0) and bright-field (numerical aperture = 0.63) condenser. The vessel image was displayed on a video monitor using a Newvicon camera, and internal diameter was measured manually with a video microometer. Pressure recordings were digitized using a Macintosh 8100/80 computer and MIO-16x data acquisition card (National Instruments; Austin, TX). Experiments were videotaped using a videotimer signal so that arteriolar diameter transients could be measured at high time resolution (1/5 real time) during replay of the videotape. To synchronize diameter and pressure measurements on replay, a video multiplexer was used to record pressure onto the audio track of the videotape. The analog output from the multiplexer was redigitized in synchrony with the replayed diameter signal.

**Servo control of pressure and wall tension.** To provide computer control over the pressurization system, the following modifications were made. Two static pressure reservoirs used for initial equilibration of the vessels were connected through three-way valves to Ling vibration pumps (model V203; Herts, UK). These pumps were driven by custom-made power amplifiers and allowed servo control of vessel pressure. Pressures at the pumps were measured using Statham P23 Db pressure transducers connected to a custom-built carrier amplifier. Output from this unit was compared with a command pressure from the computer by a custom-built servo amplifier. In initial experiments, tuning low-pass filters on each pressure channel optimized the frequency response and gain of the system. The response was confirmed to be flat to at least 20 Hz by servo-null measurements of pressure at the vessel midpoint.

**Protocols.** After equilibration and development of spontaneous myogenic tone under pressurization to 44 mmHg from the static reservoir, pressure control was switched over to the computer and set to 40 mmHg. Software programs to control this and subsequent protocols were written using LabView (National Instruments). After verification that the system was working properly, i.e., that there were no leaks and no oscillations in the servo-control system, at least one 2-min pressure step to ~90 mmHg was performed while recording internal diameter to check for myogenic constrictions. Pressure was then returned to 40 mmHg, and the preparation was allowed to stabilize for 10–15 min. 2As developed ~43% basal tone at 40 mmHg (Table 1), which is greater than that previously reported (see Fig. 3d in Ref. 4). Of 17 arterioles attempted, one was discarded for failing to develop myogenic tone, and one was discarded due to excessive spontaneous vasomotion.

After these tests, isotonic release protocols were initiated. For this, the computer system was switched from pressure control to tension control mode. The wall tension at the
control pressure (either 40 or 90 mmHg) was used as a reference point (i.e., 1.0). Wall tension was calculated from:

\[ \text{Tension} = \text{pressure} \times \text{internal radius} \quad (1) \]

While internal diameter was measured, tension was then "released" to 0.8, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 of control tension for a 2- to 15-s period. Any diameter changes during this time resulted in subsequent adjustments in pressure by the computer to maintain constant tension. Tension was then returned to control, the system switched to pressure control mode, and the vessel allowed to regain tone. After this, tension control was switched on again, and the sequence was repeated for the other afterload. Five-minute reequilibration periods were allowed between releases. Between 3 and 12 isotonic releases were performed per vessel, with the sequence of releases being determined pseudo randomly. Attempts were usually made to repeat at least two releases to 0.2 and 0.1, because the velocities of shortening at these values would have the greatest influence over subsequent determinations of maximal shortening velocity.

After a complete series of releases was determined at the first equilibration pressure (40 or 90 mmHg), pressure was set to the other level, and the vessel was allowed to reequilibrate for 20–30 min. In about half of the experiments, the order of these protocols (i.e., 40 or 90 mmHg) was reversed. At 90 mmHg, this group of 2As developed 54% myogenic tone (compare to Fig. 3d in Ref. 4). Further elevations in pressure (e.g., to 118 mmHg) resulted in only 3–5% additional tone and sometimes caused irreversible damage to these vessels (4); therefore, 90 mmHg was considered a safe pressure that would produce nearly maximal myogenic tone. In several arterioles, norepinephrine (NE) was then added to the bath after the vessel had equilibrated at 40 mmHg, and another series of isotonic release protocols were performed. The dose of NE (10 K 5–10 K 7 M) was titrated to produce approximately the same level of constriction that would be produced at 90 mmHg (see Table 1). The protocols were terminated if the vessels failed to regain myogenic tone after isotonic releases were performed.

**Off-line analysis.** The typical diameter response to an isotonic release was a rapid (<200 ms), initial collapse of the arteriole, presumably reflecting recoil of VSM series-elastic elements (23). This was followed by a period of force development (constriction) that lasted 1–10 s. After this, some vessels would begin to myogenically dilate, but at some isotonic release levels, the pressure required to control the desired level of tension was too low (<20 mmHg) to elicit a myogenic response (see Fig. 3d in Ref. 4). Accurate measurements of the diameter changes during the first few seconds following isotonic release were critical for calculation of initial isotonic shortening velocity. Videotapes of the experiment were replayed off-line in slow motion so that the diameters could be remeasured using the video micrometer (several times in some cases) and the computer could subsequently digitize its output. The replayed diameter records were used for subsequent data analysis, whereas the initial (on-line) diameter measurements were necessary for control of wall tension. However, there was almost always very close agreement between the two. Automated diameter tracking devices did not prove sufficiently accurate to track internal diameter because any glitches in tracking led to large spikes in the servo-controlled pressure system. Any errors in the online measurement would obviously generate errors in the pressure servo. However, pressure compensations were typically rather small (a few mmHg, as shown in Fig. 2), and associated errors would not have had a major effect on the shortening velocity measurements.

The diameter data for the first 1–2 s following each isotonic release (and the associated series-elastic recoil) were fit with a first-order polynomial using a least-squares algorithm to estimate isotonic shortening velocity immediately after the release. The data obtained in each release protocol were then compiled and fit to the Hill equation (23, 26). Maximum shortening velocity (V_max) was estimated from the y-intercept of these plots. We use the term “V_max” to indicate that the arterioles in our experiments were not maximally activated nor set to the optimal length for maximal isometric force development. Igor (Wavemetrics; Lake Oswego, OR) macros were used to perform analyses and to construct subsequent force-velocity plots.

**Normalization procedures.** V_max was expressed as shortening in micrometers per second or in lengths per second. For the latter case, the actual change in diameter was normalized to L_o for that vessel.

To compare force-velocity data from the three groups of vessels (P40, P90, NE), the force (tension) values must be normalized to 2A maximal isometric tension. To do this, the peak value of maximal isometric tension (T_max) for hamster cheek pouch 2As was obtained from previous measurements by Davis and Gore (4), because it was not feasible to perform this measurement on each vessel after isotonic release protocols. This value averaged 680 dyn/cm at L_o = 1.0. The minimal diameter to which 2As can constrict (0.2 of L_o) was estimated from studies by VanBavel et al. (Fig. 2 in Ref. 25) and from Jackson and Duling (Ref. 13). Given these parameters, the predicted linear relationship between maximal active tension and L_o for these 2As is shown by the dotted line in Fig. 1, according to the following equation:

\[ T_{\text{max}} = [850 \frac{\text{dyne}}{\text{cm} \cdot (D/L_o)}] - 150 \frac{\text{dyne}}{\text{cm}} \quad (2) \]

### Table 1. Baseline parameters for 2As in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Passive Diameter, μm</th>
<th>Basal Diameter, μm</th>
<th>%Tone</th>
<th>Fractional Tension</th>
<th>%Passive Tension at Basal Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>P = 40 mmHg</td>
<td>15</td>
<td>42.0 ± 2.8</td>
<td>24.4 ± 2.8</td>
<td>42.5 ± 4.2</td>
<td>0.23 ± 0.02</td>
<td>5.5 ± 2.4(5)</td>
</tr>
<tr>
<td>P = 90 mmHg</td>
<td>15</td>
<td>45.7 ± 3.2</td>
<td>19.8 ± 2.1</td>
<td>54.2 ± 3.2</td>
<td>0.55 ± 0.05</td>
<td>1.8 ± 1.1(5)</td>
</tr>
<tr>
<td>P = 40 mmHg*</td>
<td>4</td>
<td>47.4 ± 7.2</td>
<td>35.7 ± 7.7</td>
<td>25.3 ± 9.9</td>
<td>0.22 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>P = 90 mmHg*</td>
<td>4</td>
<td>47.4 ± 7.2</td>
<td>24.5 ± 4.5</td>
<td>48.4 ± 5.2</td>
<td>0.56 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>4</td>
<td>48.0 ± 6.9</td>
<td>25.4 ± 5.6</td>
<td>48.1 ± 5.3</td>
<td>0.25 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SE; n, number of arterioles; P, pressure; 2As, second-order arterioles. Passive diameter (L_o) was determined at P = 40 mmHg as described in text. Percent tone was calculated from 100 — (basal diameter/L_o)-100 on a vessel-by-vessel basis. Fractional tension was calculated from T/T_max on a vessel-by-vessel basis, where T_i is initial tension and T_max is estimated maximal active tension at the same diameter used to determine T_i. Fractional tension is analogous to “tone” as defined by VanBavel et al. (25). Parentheses in %Passive Tension at Basal Diameter indicate number of vessels analyzed for this parameter. *Subset of larger group above in which the norepinephrine (NE) protocol was also performed.
where $T_{\text{max}}$ is maximal active tension at any length (diameter) and $D_i$ is the initial vessel diameter just before isotonic release. To normalize the data for each release, the initial wall tension ($T_i$) (Fig. 1) was divided by the calculated $T_{\text{max}}$ at the respective initial diameter. This assumes negligible passive tension, as indicated in Table 1. Force values for each release were normalized in this way and used for the force-velocity determinations in Figs. 7 and 8.

**RESULTS**

Myogenic constriction represented on a tension-radius plot. One goal of these experiments was to test whether increased myogenic tone is associated with an enhanced activation of arteriolar smooth muscle. The first indication that this occurs is apparent in the myogenic behavior of a representative 2A as plotted on a length-tension curve (Fig. 1). The raw pressure and diameter data are shown as a function of time following a pressure step from 40 to 90 mmHg (see inset). The open arrows (Fig. 1) in the center of the diagram illustrate the time-dependent sequence of the myogenic constriction. At pressure equalling 40 mmHg, the radius equaled 0.6 $L_o$ (point 1). When pressure was rapidly increased to 90 mmHg, the vessel distended to a radius of 0.68 $L_o$ (point 2), then constricted to a minimum of 0.4 $L_o$ (point 3), and maintained a steady-state constriction at that diameter. For reference purposes, the passive radius-tension relationship for the same 2A is also plotted (open circles) as is the maximal active tension curve (dashed line) calculated from Davis and Gore (4). It is apparent that the myogenic constriction is associated with a leftward shift of the radius-tension relationship for the arteriole. The solid arrows in Fig. 1 indicate the relative degree of myogenic tone, where tone is defined as the difference between the passive diameter at 40 mmHg ($L_o$) and the normalized steady-state diameter ($D/L_o$). Thus this 2A developed ~40% tone at a pressure equalling 40 mmHg and ~60% tone at a pressure equalling 90 mmHg. Also shown in Fig. 1 are vertical lines that project from the two steady-state diameters to denote the intersections with the maximal active tension curve. The ratio of $T_i$ to $T_{\text{max}}$ at each diameter may perhaps be a superior index of “tone,” as defined by VanBavel et al. (25). However, in most previous myogenic studies, the term “tone” refers to a difference in the active versus passive diameter. Therefore to avoid confusion, we employ the term “fractional tension” to refer to the $T_i$-$T_{\text{max}}$ ratio under different conditions. With the use of this criterion, the 2A shown in Fig. 1 had a fractional tension equalling 0.19 at 40 mmHg and 0.49 at 90 mmHg, respectively. The average values for tone and fractional tension at both pressures and after NE are summarized in Table 1.

A leftward shift in the length-tension relationship in response to pressure elevation suggests increased arteriolar smooth muscle activation during myogenic tone development; however, more rigorous experimental evidence would be provided by demonstration of an increase in $V_{\text{max}}$. $V_{\text{max}}$ can be determined from measurements of isotonic shortening velocity using isotonic release protocols (23) in which the muscle is quickly released from a predetermined afterload to a series of fractional afterloads, during which shortening velocity is measured at each step. This procedure is typically performed on maximally activated muscle, but the resulting force-velocity relationship is also valid for intermediate activation states (2, 19). Because this procedure had never before been performed on myogenically active arterioles, it was first necessary to validate its feasibility.

Isotonic release protocol. Changes in pressure and diameter as a function of tension during two isotonic releases are shown in Fig. 2. The baseline diameter of this arteriole was 24–27 µm at a pressure equalling 40 mmHg. In response to a sudden drop in wall tension from the calculated control value to 20% of control, there was a rapid initial collapse of the vessel (<0.2 s), which presumably represented the recoil of series elastic components (SEC) (26). After the recoil was a 2- to 3-s shortening phase until the arteriole suddenly began to dilate at ~1.8 s after release. The myogenic dilation (induced by the low pressure required to reduce afterload to 0.2) was variable in magnitude and time of onset from vessel to vessel, but never interfered with accurate determinations of the rate of initial
shortening phase commences, lasting corresponds to recoil of the series elastic component. After this, a and tension is associated with a rapid (/<100 ms) fall in tension that to 20% of its control value (/H11002). Shortly after this switch, tension is suddenly released to 50 mmHg, a second release was performed on this vessel, to afterload equalling 0.6. As can be seen from Fig. 2, right, the phase of passive collapse is not clearly discernable, but the initial rate of shortening is lower than for the previous release. Also, the magnitude of the secondary myogenic dilation is greater (dilating above the control diameter; Fig. 2, left) presumably because the vessel is more myogenically active at the pressure required to achieve an afterload of 0.6 (~30 mmHg) compared with the pressure required to achieve an afterload of 0.2 (~15 mmHg).

Repeatability of diameter changes during identical isotonic releases. To verify that reliable and repeatable changes in diameter could be recorded during isotonic releases, a series of nearly identical release protocols were repeated on a single 2A, as shown in Fig. 3. This arteriole was allowed to develop myogenic tone at a pressure of 40 mmHg. The servo system was switched to tension control mode, and the initial computed wall tension was used as a reference point. Tension was then released to an afterload equivalent to 20% of this value for ~14 s while recording diameter (in slow motion on video playback). The servo system was switched off, pressure was returned to 40 mmHg, and the vessel was allowed to regain myogenic tone. This procedure was repeated two times. The three resulting releases were then superimposed for comparison. The time courses of the diameter changes following isotonic release are remarkably consistent, especially over the first 2–3 s after release (Fig. 3). Note that there are some differences in the baseline tension for each trial due to slight variations in the amount of myogenic tone developed during each recovery period. Also, note that a partial myogenic relaxation occurred (t = 9 s) in one of the trials but that it was inconsequential to the measurement of initial shortening velocity. Importantly, the absolute diameter changes during the isotonic shortening phase (2.0–2.5 μm) were well within the resolution of the microscope system.

Analysis of data from a series of isotonic releases. To assess the force-velocity relationship for each arteriole, tension was released to a series of different afterloads. Each release, upon completion of the force change and SEC recoil, was associated with a shortening phase in which the rate of shortening slowed progressively with time (Figs. 2 and 3). When the logarithm of (1 – fractional shortening) was plotted against time for isotonic releases to various afterloads, the data for the first 2 s of each shortening phase were almost always well fit by a first-order exponential function (not shown).

Figure 4A shows the complete diameter changes for this series of isotonic releases (4 additional releases for shortening. After reequilibration for about 1.5 min at a pressure equalling 40 mmHg, a second release was performed on this vessel, to afterload equalling 0.6. As can be seen from Fig. 2, right, the phase of passive collapse is not clearly discernable, but the initial rate of shortening is lower than for the previous release. Also, the magnitude of the secondary myogenic dilation is greater (dilating above the control diameter; Fig. 2, left) presumably because the vessel is more myogenically active at the pressure required to achieve an afterload of 0.6 (~30 mmHg) compared with the pressure required to achieve an afterload of 0.2 (~15 mmHg).

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Figure 4A shows the complete diameter changes for this series of isotonic releases (4 additional releases for
this vessel are not shown for clarity). Releases to progressively lower afterloads were associated with progressively greater recoil of the SEC and progressively higher rates of initial shortening. Again, some slight variations occurred in the amount of myogenic tone regained between release protocols, leading to slight variations in the initial tension. Trials in which vessels did not regain sufficient tone to recover to within 15% of their initial wall tension were not included in the analysis; to do so might have skewed the data by combining data from vessels that had significantly different degrees of myogenic tone.

Shortening velocities were measured from linear fits of the diameter data points over the initial 1–2 s after SEC recoil. The set of calculated shortening velocities for each vessel were then fit, using a nonlinear curve-fitting procedure, to the hyperbolic form of the Hill equation to obtain coefficients for extrapolation of the maximum shortening velocity at zero force on a standard force-velocity plot. The form of the Hill equation used was

$$\frac{(T + a) - (V + b)}{T_1 + a} = \frac{(T_1 + a) - b}{H}$$

where $T$ and $V$ are the relative afterload (tension) and velocity, respectively, and $a$ and $b$ are constants corresponding to the $y$- and $x$-intercepts, respectively, of a rectangular hyperbola. $V_{\text{max}}$ for this vessel was determined from the $y$-intercept of this equation. Figure 4, B and C, shows the force-velocity plot of the data for the vessel in Fig. 4A, expressed in terms of shortening in micrometers per second (Fig. 4B) and lengths per second (Fig. 4C).

Comparison of active and passive tensions. The above procedure is commonly used to determine unloaded shortening velocity on isolated strips of muscle. A possible concern with using it to analyze isotonic shortening data from arterioles, which contain significant amounts of nonforce-generating connective tissue, would be if passive tension made a significant contribution to total wall tension under these conditions and thereby contaminated measurements of active shortening. To address this issue, we compared the range of vessel radius changes that occurred during isotonic releases with the passive tension-radius relationship obtained for each vessel at the end of the experiment, when active tone had been eliminated by calcium-free bath solution containing nitroprusside. An example of this analysis is shown in Fig. 5, where the open circles represent the passive tension-radius relationship and some of the raw isotonic release data for the same vessel are plotted as individual points.

![Fig. 4. Method of data analysis is illustrated. A: diameter changes for 5 isotonic releases (as recorded on playback of the videotape in slow motion and with corrected time scales). Note progressive increase in magnitude of SEC recoil and progressive increase in initial velocity of shortening as afterload is released to smaller fractions of control tension. Possible exceptions to the latter statement are the very similar initial velocities for releases to 0.1 and 0.3. Myogenic dilations of various magnitudes occur at time $t_{11}$ s for three of the releases. Four other releases were performed on the same vessel (data not shown for clarity). B: force-velocity plot of nonnormalized data for all 9 isotonic releases. These data were fit to find the Hill coefficients, $a$ and $b$ (see text for details), and the coefficients were then used to draw a hyperbolic curve through the data and to determine maximal velocity ($V_{\text{max}}$) from the $y$-intercept. C: force-velocity plot of same data after normalization of shortening velocity to $L_o$ for this vessel. Lengths/s = ($\mu$m·s$^{-1}L_o^{-1}$)·100.](http://ajpheart.physiology.org/)
myogenic tone (pressure = 90 mmHg; n = 12). For this analysis, the data were pooled by afterload, the means were calculated, and then Hill coefficients were computed from nonlinear fits of the transformed data. There is clear separation between the data sets, as indicated by comparisons of the group data at each afterload. Shortening velocities at the three lowest afterloads, 0.3, 0.2, and 0.1, were all significantly higher in arterioles with higher initial myogenic tone (ANOVA, P < 0.05). The curves through the data sets represent the best fits of the data to the Hill equation. Determinations of \( V_{\text{max}} \) from the \( y \)-intercepts suggest that \( V_{\text{max}} \) is substantially higher for arterioles with higher myogenic tone.

Two additional analysis methods were also used to examine this data set, as shown in Table 2. First, raw or normalized (to \( L_o \)) shortening velocities for individual vessels were fit to the Hill equation, and \( V_{\text{max}} \) for each vessel was estimated from the \( y \)-intercepts of the force-velocity plots. These values were then statistically analyzed to obtain an average \( V_{\text{max}} \) for each of the groups. Second, all of the data points for each group were fit to the Hill equation, and \( V_{\text{max}} \) was estimated from the \( y \)-intercept of each force-velocity plot. As shown in Table 2, the two alternative analyses confirmed that \( V_{\text{max}} \) was higher in vessels with higher initial myogenic tone.

**Analysis of data after normalization to maximal isometric force.** As mentioned previously, force-velocity data are typically normalized to some force index such as maximal isometric tension. To do this, each data point was normalized to maximal active tension at the respective diameter, as diagrammed in Fig. 5. For example, from the point at which isotonic releases were performed at a pressure of 90 mmHg, this arteriole had...
58% initial tone (1.0–0.42), $T_0 = 78$ dyn/cm, an estimated maximal active tension of 135 dyn/cm (from Eq. 2), and therefore a fractional tension of 0.58. The vertical open arrow in Fig. 5 indicates the release phase and the horizontal open arrow indicates the shortening phase when tension was released to 10% of its initial value. Single releases to other afterloads for the pressure at 40 mmHg and NE protocols are also shown to indicate the representative relationships among active and passive tension, tone, and fractional tension relative to the dimensional changes that occurred during the isotonic releases. It should be clear that passive tension is minimal during the isotonic releases.

When all data points for the 40- and 90-mmHg pressure data sets were normalized to maximal tension in this manner, the force-velocity curves appear as shown in Fig. 7. Nonlinear fits of the two data sets to the Hill equation yielded $V_{\text{max}}$ estimates of 4.2 and 8.3 lengths/s for the 40- and 90-mmHg pressure data sets, respectively. The intersection of the 40-mmHg data set with the force axis occurred at a fractional tension of 0.22, whereas the 90-mmHg data set intersected at a fractional tension of 0.56. These values were the average $T/T_{\text{max}}$ values before release for the two groups, and the curve-fitting parameters ($T_i$ in Eq 3) were adjusted to force the $x$-intercept at these respective values. The approximate twofold higher $V_{\text{max}}$ associated with vessels at higher pressure is consistent with the raw data analyses shown in Fig. 6 and Table 2.

Comparison of force-velocity plots for vessels with and without NE. To test whether agonist-induced activation of arteriolar smooth muscle would produce a similar shift in the force-velocity relationship, we studied a third group of vessels treated with NE at a pressure of 40 mmHg. The concentration of NE was titrated to produce an additional constriction roughly comparable to the level of myogenic tone seen at a pressure of 90 mmHg. These data, after normalization to maximal active tension, are shown in Fig. 8, along with the data set for arterioles equilibrated at the same pressure in the absence of NE. The curves represent the best fits of the two data sets to the Hill equation. Shortening velocities at almost all afterloads were significantly higher for arterioles constricted with NE, and determinations of $V_{\text{max}}$ from the $y$-intercepts suggest that $V_{\text{max}}$ was substantially higher for arterioles treated with NE. For the NE data, the average initial $T/T_{\text{max}}$, and hence the force intercept, was at fractional tension equaling 0.26.

**DISCUSSION**

The results of our study constitute the first experimental evidence that increases in myogenic tone reflect an enhanced $V_{\text{max}}$ of VSM. Although previous studies of arteriolar mechanics (13, 15) and of myogenic signaling pathways (5, 25) indicate that arteriolar smooth muscle is indeed activated by pressure, $V_{\text{max}}$ is perhaps the best quantitative parameter by which to assess the degree of this activation. $V_{\text{max}}$ has not previously been measured in true arterioles. In addition to a shift in $V_{\text{max}}$ with pressure, NE-induced tone was also associated with an increase in $V_{\text{max}}$; however, the characteristics of the two respective force-velocity curves were different. Myogenic tone produced roughly parallel increases in velocity and force, whereas NE produced increases in velocity at low force. Therefore, myogenic and NE-induced activation of arteriolar smooth muscle are associated with different mechanical states of ar-

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**Table 2. Summary of analysis methods to determine $V_{\text{max}}$**

<table>
<thead>
<tr>
<th>Analysis Method</th>
<th>$P = 40$ mmHg</th>
<th>$P = 90$ mmHg</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{\text{max}}$, μm/s</td>
<td>$V_{\text{max}}$, len/s</td>
<td>$n$</td>
</tr>
<tr>
<td>Pooled by afterload</td>
<td>1.62</td>
<td>3.86</td>
<td>12</td>
</tr>
<tr>
<td>Average of individual fits</td>
<td>1.64 ± 0.22</td>
<td>4.16 ± 0.47</td>
<td>13</td>
</tr>
<tr>
<td>Group fit</td>
<td>1.59</td>
<td>3.79</td>
<td>108</td>
</tr>
</tbody>
</table>

Errors are means ± SE as appropriate. Lengths per second (Len/s) values are calculated as μm·s⁻¹·L⁻¹.100. Data in “Pooled by afterload method” are shown in Fig. 6. Each “Average of individual fits” data set for each vessel was fit according to METHODs and the average of the maximal velocity ($V_{\text{max}}$) values were then calculated. All “Group fit” data for each of the groups were fit to the Hill equation.
arteriolar smooth muscle, which is likely a reflection of different underlying signaling pathways activated by the two stimuli.

Consideration of technical issues. Our conclusions are subject to consideration of several technical issues related to the unique way in which these experiments were performed. One issue is the accuracy of diameter measurements. Because the diameter changes that occur during isotonic releases are only a few microns in magnitude, we chose an objective/condenser system based on the compromise between optical quality and working distance. Given the objective, condenser, and wavelength of light used in our system, the theoretical resolution of this system is 0.38 μm (see Ref. 12, p. 115). Of course, light scattering above and below the focal plane raises this value, an effect that can be rather large when visualizing arterioles in intact tissue (12). However, the use of an isolated arteriole, in the absence of parenchymal tissue and adventitia, minimizes degradation of the image due to light scattering. We estimate that diameter changes of 0.5 μm could clearly be resolved under the conditions of our experiments (4). Moreover, for determination of V_{max}, the most critical measurements of shortening velocity are those required for isotonic releases to the lowest afterloads, e.g., 0.1 and 0.2, and it is at these afterloads that the absolute magnitude of shortening is greatest, usually well over 0.5 μm and sometimes over 2 μm (Figs. 2–4). Therefore, we have the most confidence in accurate diameter measurements for the most critical protocols.

One problem with estimating V_{max} is that maximal isometric tension should, ideally, have been determined for each 2A at the end of each experiment to normalize force; however, this was simply not feasible given the complexity of our protocols. Davis and Gore (4) have previously discussed the difficulties involved in determining maximal isometric tension in small arterioles, and their study is the only one of its kind. Given the difference in size between the “2As” (L_o = 58 μm) studied by Davis and Gore (4) and the 2A used for the present study (L_o = 42 μm), our estimate of maximal active tension may be high. Likewise, errors in our estimate of 0.2 L_o/L_{max} as the zero active tension intercept on the maximal tension-length curve would alter the normalization of all of the force data in Figs. 7 and 8. However, inaccuracies in our estimates of both of parameters would simply lead to roughly parallel shifts in normalized force values for all three groups (P40, P90, and NE) along the force axis, and thus would not fundamentally alter our conclusions about differences between the groups.

Another potential concern with our analysis relates to possible differences in the initial length of VSM in the two groups (pressure = 40 vs. 90 mmHg). Initial length is an important determinant of V_{max}, with V_{max} being highest at the optimal length of the muscle, regardless of the level of muscle activation at which the force-velocity relationship is determined (19, 23). For example, in small arteries, V_{max} increased by threefold with length increases from 0.4 to 0.7 of maximal length.

Comparison with previous measurements of V_{max}. How do our estimates of V_{max} compare with measurements from other smooth muscle? Comparisons are necessarily limited by the fact that we measured V_{max} of partially activated arteriolar smooth muscle, whereas most other studies have measured V_{max} of maximally activated smooth muscle. We obtain V_{max} values between 0.04 and 0.08 lengths/s for arterioles with low and high myogenic tone, respectively (Table 2). Estimates of V_{max} for other smooth muscles are 0.61 lengths/s in the Bufo stomach (26), 0.51–0.53 lengths/s in the rat portal vein (14, 21), 0.12 lengths/s in the hog carotid (11), and 0.013 lengths/s in the rat caudal vein (20). With the exception of the latter study, our estimates of V_{max} for arterioles are, predictably, at the low end of the reported range. However, in the most directly relevant study, Boels et al. (1) reported a V_{max} of...
0.041 lengths/s for coronary “microarteries” (140 µm ID), which is reasonably close to ours (Table 2). However, the unloaded shortening velocity for maximally activated 2As remains to be determined.

Comparison of effects of myogenic- versus agonist-induced tone. Our results show that high levels of myogenic tone and NE produce comparable increases in $V_{max}$. However, pressure-induced tone (Fig. 7) is associated with smaller increases in velocity (4.2 to 8.3 lengths/s; ~100% increase) than force (0.22 to 0.56 $T_i/T_{max}$; ~250% increase), whereas NE-induced tone (Fig. 8) is associated with larger increases in velocity (4.2 to 7.5 lengths/s; ~80% increase) than force (0.22 to 0.26 $T_i/T_{max}$; ~20% increase). The differences likely reflect underlying differences in the mechanical states produced by pressure and NE. The classical interpretation of the force-velocity relationship is that an increase in isometric force represents an increase in the number of active cross bridges, whereas an increase in $V_{max}$ represents an increase in the cycling rate of active cross bridges (19). Thus the differences in the force-velocity curves produced by pressure and NE lead to the conclusion that myogenic tone is associated with an increase in both the number and cycling rate of active cross bridges.

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