Muscle Length, Shortening, Myoplasmic \([\text{Ca}^{2+}]\), and Activation of Arterial Smooth Muscle

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The effect of muscle length on smooth muscle contraction was evaluated by measuring myoplasmic \([\text{Ca}^{2+}]\) (with aequorin), myosin light chain phosphorylation, length, and isometric stress in histamine-stimulated swine carotid media preparations. Tissues were equilibrated at the optimal length for stress development \((L_o)\). Isometric contractions at short tissue lengths \((0.7 \times L_o)\) were associated with a decrease in maximal stress development. Isometric contraction at \(0.7 \times L_o\) also reduced the sensitivity to histamine as measured by steady-state increases in \([\text{Ca}^{2+}]\), phosphorylation, or stress. This suggests that decreased agonist sensitivity at shorter lengths is caused by reduced \([\text{Ca}^{2+}]\) mobilization. Isotonic shortening also led to decreases in histamine sensitivity. Isometric contractions at \(1.2 \times L_o\) were not associated with significant changes in histamine-induced increases in \([\text{Ca}^{2+}]\). The [\(\text{Ca}^{2+}\)] dependence of phosphorylation was not altered at \(0.7\) or \(1.2\) \(L_o\). Sinusoidal length changes from 0.95 to 1.05 \(L_o\) at 1 Hz were not associated with significant changes in the resting or histamine-stimulated \([\text{Ca}^{2+}]\). These results suggest that \([\text{Ca}^{2+}]\) mobilization and the resulting contraction is relatively independent of length changes near \(L_o\). Inactivation occurs at lengths substantially below \(L_o\) where \([\text{Ca}^{2+}]\) mobilization by agonists is impaired. (Circulation Research 1990;66:1354–1361)

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mooth muscle performs two roles: 1) maintenance of tone and 2) change in volume of hollow organs. The latter may involve substantial changes in tissue length, whose effects on myoplasmic \([\text{Ca}^{2+}]\) and crossbridge function are not well understood. \([\text{Ca}^{2+}]\)-dependent crossbridge phosphorylation appears to be the primary regulator of shortening velocity and stress production in smooth muscle.1–3 Our goal was to evaluate the effect of vascular smooth muscle cell length or shortening on myoplasmic \([\text{Ca}^{2+}]\), myosin phosphorylation, and the mechanical response.

Shorter muscle lengths in smooth muscle are associated with decreases in the sensitivity to agonist stimulation.4,5 Potentially, this decrease in sensitivity could be caused by length-dependent decreases in the myoplasmic \([\text{Ca}^{2+}]\) mobilized by the agonist. Alternatively, the \([\text{Ca}^{2+}]\) sensitivity of the system(s) regulating crossbridges may be dependent on length.

There is no length sensitivity to changes in extracellular \([\text{Ca}^{2+}]\); however, this does not rule out a length-dependent effect on intracellular \([\text{Ca}^{2+}]\). Changes in tissue length are known to affect mobilization of myoplasmic \([\text{Ca}^{2+}]\) in skeletal muscle.8 Gunst9 evaluated the length dependence of aequorin-estimated myoplasmic \([\text{Ca}^{2+}]\) in the electrically stimulated canine trachealis. Shorter lengths were associated with higher levels of myoplasmic \([\text{Ca}^{2+}]\) in response to field stimulation. If myoplasmic \([\text{Ca}^{2+}]\) mobilized by contractile agonists has a similar length dependence, the decreased agonist sensitivity observed by Price et al.4,5 cannot be explained by length-dependent reductions in \([\text{Ca}^{2+}]\).

Bárány et al.10 reported that rapid stretching (more than 30%) of helically cut swine carotid preparations was associated with transient increases in myosin phosphorylation. Force redeveloped after stretched tissues were shortened, showing that the increased myosin phosphorylation could support a contraction. No changes in fura-2–estimated myoplasmic \([\text{Ca}^{2+}]\) occurred with small releases and stretches of rabbit pulmonary artery or guinea pig ileum.11 However, stretches of guinea pig ileum occasionally induced a small increase in \([\text{Ca}^{2+}]\) and force after a delay of a few seconds.12 Gunst13 reported that prior shortening was associated with depression of shortening velocity. Potentially this depression may be caused by changes in \([\text{Ca}^{2+}]\).

We hypothesized that short tissue length or shortening attenuates agonist-induced increases in intra-
cellular [Ca^{2+}], thus explaining the decrease in agonist sensitivity. Additionally, we hypothesized that stretching smooth muscle increases intracellular [Ca^{2+}] and produces stretch-induced increases in myosin phosphorylation and force.

Materials and Methods

Swine common carotid arteries were obtained from a slaughterhouse and transported at 2°C in physiological salt solution (PSS). Dissection of medial strips and mounting were performed as illustrated by Driska et al.\(^\text{14}\) The intimal surface was mechanically rubbed in a manner previously shown to remove the endothelium. Changes in tissue length (expressed as a fraction: \(L/L_\text{o}\), where \(L\) is tissue length and \(L_\text{o}\) is the optimal length for stress development) induce proportional changes in cell length in the preparation.\(^\text{15}\)

PSS contained (mM) NaCl 140, KCl 5, 3-(N-morpholino)propanesulfonic acid 2, CaCl\(_2\) 1.6, MgCl\(_2\) 1.2, Na\(_2\)HPO\(_4\) 1.2, and d-glucose 5.6 (pH adjusted to 7.4 at 37°C). Agonist stimulation was performed by injecting an appropriate volume of 10 mM stock histamine into the tissue bath. Agonist stock solutions were prepared daily.

Aequorin (batch 2, obtained from Dr. John Blinks, Mayo Medical School, Rochester, Minnesota) was loaded by iso-osmotic hyperpermeabilization as described by Rembold and Murphy.\(^\text{3}\) The loading procedure did not affect maximal stress development or the time course of myosin phosphorylation.\(^\text{3,16,17}\)

\(L_\text{o}\) was determined by a modification of the method of Herlihy and Murphy\(^\text{18}\) in which \(L_\text{o}\) was estimated before contraction to avoid attenuation of aequorin signals with repeated contractions.\(^\text{3}\) Aequorin-loaded tissues were stretched repeatedly until the sustained stress (after stress relaxation) was approximately \(1.0 \times 10^5\) N/m\(^2\). The tissue was then shortened approximately 10% to give a passive stress (\(S_\text{p}\)) of \(0.1 \times 10^2\)–\(0.2 \times 10^2\) N/m\(^2\), and length was measured. When the tissue was contracted maximally, the increase in stress over passive stress (i.e., active stress [\(S_a\)]) typically \(1 \times 10^2\)–\(2 \times 10^2\) N/m\(^2\) was measured. \(L_\text{o}\) was calculated with the formula \(L/L_\text{o} = 1.155 + 0.154 \log S_a/S_\text{p}\). This formula is based on length-tension studies in the swine carotid,\(^\text{18}\) in which it was found that \(L_\text{o}\) occurs at the length where \(S_\text{p} = 0.1 S_a\).

Light measurements were made in a light-tight enclosure that allowed simultaneous measurement of aequorin light, force, and length.\(^\text{16}\) Force was measured with a servo (model 300H, Cambridge Technology, Cambridge, Massachusetts) in the isometric or isotonic mode and was displayed on a rectilinear recorder in synchronization with the light signal. Stress was calculated as force per cross-sectional area, which was estimated from measured length, weight, and a density of 1.050 g/cm\(^3\).

Aequorin light signals are presented in the form log \(C/C_{\text{max}}\), where \(C\) is the photon count (in counts per second) and \(C_{\text{max}}\) is an estimate of the peak light intensity that would be recorded if all of the aequorin in the tissue were instantaneously exposed to 5 mM CaCl\(_2\).\(^\text{19}\) The usual expression for aequorin light production is log \(L/L_{\text{max}}\). Log \(C/C_{\text{max}}\) was adopted to avoid confusion with tissue length [L]). Aequorin light emission was calibrated in a series of Ca\(^{2+}\)-EGTA buffers with 0.5 mM Mg\(^{2+}\) at 37°C.\(^\text{3}\) Changes in log \(C/C_{\text{max}}\) and \(S_a\) were compared among groups using Student's unpaired \(t\) test, and significance was defined as \(p<0.05\). EC\(_{50}\) is the histamine concentration that produces 50% of the maximal response and was determined by sigmoidal curve fitting.

Sequential contractions attenuate aequorin signals.\(^\text{3}\) This artifact was minimized by a long interval between contractions in the experiment illustrated in Figure 6. Only the first contraction in an aequorin-loaded preparation was reported in all other data. Thus, comparisons of contractions at different lengths are, by nature, unpaired. Illustrated [Ca\(^{2+}\)] and stress data induced by 100 μM histamine stimulation at 1.0 \(L_\text{o}\) were previously published.\(^\text{17}\)

Results

The dose dependence of histamine-induced isometric contractions at various tissue lengths is shown in Figure 1. Active stress was attenuated both at 1.2 and 0.7 \(L_\text{o}\) when compared with 1.0 \(L_\text{o}\). Tissues at 0.7 \(L_\text{o}\) were also less sensitive to agonist stimulation than at 1.0 \(L_\text{o}\) (Figure 1, lower panel). The histamine dose
for half-maximal contraction was 4.0±1.7 μM at 0.7 L₀ compared with 1.6±2.0 μM at 1.0 L₀. The dose-response curve at long lengths (1.2 L₀) was similar to that observed at 1.0 L₀.

To evaluate the mechanism of the decrease in histamine sensitivity at shorter lengths, we examined histamine-induced changes in aequorin-estimated myoplasmic [Ca²⁺] during isometric contractions at 0.7 L₀. No change in aequorin-estimated myoplasmic [Ca²⁺] was observed after a quick release to 0.7 L₀ (Figure 2). Stimulation with 100 μM histamine was associated with a [Ca²⁺] transient followed by sustained elevated values (Figure 2, solid line) that were similar to those observed in a second set of tissues stimulated with 100 μM histamine at 1.0 L₀ (Figure 2, squares). Final stress values at 0.7 L₀ were slightly, but not significantly, lower than the separate set of tissues studied at 1.0 L₀.

Removal of histamine resulted in a fall in [Ca²⁺] to resting levels and relaxation. A quick stretch of these relaxed preparations back to 1.0 L₀ resulted in a large transient increase in aequorin light (Figure 2). Stress transiently increased during the stretch and then relaxed to near resting values. The stretch-induced increases in aequorin light were not associated with proportional increases in stress (compare the histamine- and stretch-induced increases in aequorin light with resulting stress in Figure 2).

Since shorter lengths were associated with a decrease in histamine sensitivity, we evaluated the

![Figure 2](https://circres.ahajournals.org/)

**Figure 2.** Graphs showing the change in length (as a fraction of the optimal length for stress development [L₀]), log C/Cₘₐₓ (intracellular [Ca²⁺]), and total stress (passive and active) observed upon a quick release from 1.0 to 0.7 L₀ at 10 minutes and stimulation with 100 μM histamine at 20 minutes. The histamine was removed from the bathing solution at 50 minutes, and the preparations were quickly stretched back to 1.0 L₀ at 60 minutes. The results are shown as solid line (mean)±1 SEM (dotted lines) with n=5. The control response in a second set of tissues to 100 μM histamine at 1.0 L₀ (values are from Reference 17) is shown in squares (mean)±1 SEM (error bars) with n=4. For any given tissue, force was 30% greater at 1.0 L₀ than at 0.7 L₀. The step changes in length were imposed through the lever system at 4 mm/sec.

![Figure 3](https://circres.ahajournals.org/)

**Figure 3.** Graphs showing the change in length (as a fraction of the optimal length for stress development [L₀]), log C/Cₘₐₓ (intracellular [Ca²⁺]), and total stress observed upon a quick release from 1.0 to 0.7 L₀ at 10 minutes and stimulation with 10 μM histamine at 20 minutes. Results are shown as solid line (mean)±1 SEM (dotted lines) with n=4. The control response to 10 μM histamine at 1.0 L₀ is shown in squares (n=9). Filled squares represent significant differences between the two responses at p<0.05, half-filled squares represent p<0.10, and open squares represent p>0.10. No data are shown for the first minute after this quick release, which was performed by closing the shutter to open the aequorin light-gathering apparatus and by adjusting the micrometer mounting of the tissue.
response to lower histamine concentrations. Isometric contractions induced by 10 \mu M histamine at 0.7 \textit{L}_o were associated with [Ca\textsuperscript{2+}] transients (Figure 3, solid line) that were slightly less than those observed in a second set of tissues at 1.0 \textit{L}_o (Figure 3, squares). [Ca\textsuperscript{2+}] and stress remained significantly above resting values in the continued presence of histamine but were significantly less than those observed at 1.0 \textit{L}_o. Myosin phosphorylation values determined 30 minutes after histamine addition were significantly less at 0.7 \textit{L}_o (0.20±0.01 mol P/mol 20-kDa light chain) than at 1.0 \textit{L}_o (0.32±0.01 mol P/mol 20-kDa light chain; \textit{p}<0.05).

Isometric contractions induced by 3 \mu M histamine at 0.7 \textit{L}_o were also associated with [Ca\textsuperscript{2+}] transients and sustained values that were significantly less than those observed in a second set of tissues at 1.0 \textit{L}_o (Figure 4). Increasing histamine concentrations produced proportional increases in [Ca\textsuperscript{2+}] and stress.

Maximal increases in [Ca\textsuperscript{2+}] did not significantly differ at 0.7 and 1.0 \textit{L}_o (Figure 2). However, increases in [Ca\textsuperscript{2+}] were less sensitive to the histamine concentration (Figures 3 and 4). The histamine dose for half-maximal increase in [Ca\textsuperscript{2+}] (measured 30 minutes after stimulation) was 7.9±2.0 \mu M at 0.7 \textit{L}_o and 3.8±3.3 \mu M at 1.0 \textit{L}_o. This decrease in sensitivity to histamine was similar to the stress data (Figure 1).

The previous experiments revealed the effect of isometric contractions at a shorter length. We also evaluated the effect of isotonic shortening. Tissues were allowed to shorten at very low loads (0.013±0.008×10\textsuperscript{3} N/m\textsuperscript{2}) to allow estimation of [Ca\textsuperscript{2+}] changes during nearly unloaded shortening. Low load shortening in the resting state was associated with a small decrease in tissue length due to passive elastic forces but not with a change in resting [Ca\textsuperscript{2+}] (Figure 5). Stimulation with 10 \mu M histamine was associated with rapid shortening to approximately 0.5 \textit{L}_o (Figure 5, tracing B). The [Ca\textsuperscript{2+}] transient was similar, but sustained [Ca\textsuperscript{2+}] was less than that observed in a prior control contraction of the same tissue at 1.0 \textit{L}_o (Figure 5, tracing A).

A quick stretch from 0.7 to 1.0 \textit{L}_o increased aequorin light without a proportional contraction (Figure 2). A quick stretch from 1.0 to 1.2 \textit{L}_o also transiently elevated the aequorin light signal (Figure 6). Myosin phosphorylation was not significantly increased by this stretch (phosphorylation values were 0.11±0.02 mol P/mol 20-kDa light chain before and 0.11±0.01 mol P/mol 20-kDa light chain at 1 minute after the stretch to 1.2 \textit{L}_o; \textit{n}=4). The aequorin light signal approached resting values after 20 minutes at 1.2 \textit{L}_o.

Long tissue lengths were not associated with a significant change in the sensitivity of tissues to histamine (Figure 1). Isometric stimulation with 10 \mu M histamine at 1.2 \textit{L}_o was associated with transient and sustained increases in [Ca\textsuperscript{2+}] (Figure 6) not statistically different from those observed in a second set of tissues stimulated at 1.0 \textit{L}_o (Figure 6, open squares). Similarly, myosin phosphorylation values 30 minutes after 10 \mu M histamine stimulation were similar at 1.2 \textit{L}_o (0.36±0.04 mol P/mol 20-kDa light chain) and at 1.0 \textit{L}_o (0.32±0.01 mol P/mol 20-kDa light chain; \textit{n}=4, \textit{t}=-0.93, \textit{p}=NS).

The tissues were then quickly shortened from 1.2 to 1.0 \textit{L}_o after 30 minutes of histamine stimulation (Figure 6). No change in [Ca\textsuperscript{2+}] was observed with this step shortening, and stress slowly redeveloped to levels comparable with those observed in the control tissues at 1.0 \textit{L}_o.

Large arteries are exposed to pulsatile changes in blood pressure and diameter. The effect of oscillatory changes in tissue length was evaluated by imposing length changes from 0.95 to 1.05 \textit{L}_o at a frequency of 1 Hz (Figure 7). Neither resting nor histamine-
induced [Ca$^{2+}$] changes were affected by these oscillatory length changes.

We tested whether length affected the [Ca$^{2+}$] dependence of phosphorylation during isometric contractions. Myosin phosphorylation values were measured 30 minutes after 3, 10, and 100 μM histamine stimulation at 0.7 L$_{0}$ and 10 and 30 minutes after 10 μM histamine stimulation at 1.2 L$_{0}$. The [Ca$^{2+}$] dependence of phosphorylation was not significantly altered by muscle length (Figure 8), although a small decrease in the [Ca$^{2+}$] dependence of phosphorylation at 1.2 L$_{0}$ may occur.

Increases in cross-sectional area associated with shortening could potentially attenuate the light signal after 10 μM histamine stimulation at 1.2 L$_{0}$.

**Figure 5.** Graphs showing the change in length (L) (as a fraction of the optimal length for stress development [L$_{opt}$]), log C/C$_{max}$ (intracellular [Ca$^{2+}$]), and total stress upon release to a very light load. Tracing A is the control isometric tracing in which the tissue is held at 1.0 L$_{0}$ throughout 30 minutes of 10 μM histamine stimulation. The histamine was then washed out for 2.5 hours, and the tissue was released at 10 minutes to allow shortening at a very light load (tracing B). Histamine (10 μM) was added at 40 minutes, and the resultant shortening and light signal were observed. Tracings A and B are superimposed such that the histamine stimulation occurs at the same time. This experiment is representative of three other experiments.

**Figure 6.** Graphs showing the change in length (as a fraction of the optimal length for stress development [L$_{opt}$]), log C/C$_{max}$ (intracellular [Ca$^{2+}$]), total stress, and active stress (corrected for changes in passive stress due to changes in length) observed with a quick stretch from 1.0 to 1.2 L$_{0}$ at 10 minutes, stimulation with 10 μM histamine at 30 minutes, and a quick release to 1.0 L$_{0}$ at 60 minutes. The results are shown as a solid line (mean) ±1 SEM (dotted lines) with n=4. The control response to 10 μM histamine at 1.0 L$_{0}$ is shown in squares (n=9, replotted from Figure 3). Filled squares represent significant differences between the two responses at p<0.05, and open squares represent p>0.10. No data are shown for the first minute after the quick stretch. The quick release to 1.0 L$_{0}$ was performed by electrically changing the length through the lever system. In this figure, total stress is the force per area measured by the lever servo system. Active stress was calculated by subtracting passive stress at the appropriate length.
emanating from the center of the smooth muscle preparation. However, only the sustained response to lower histamine doses was attenuated. Resting [Ca$^{2+}$], both before and after histamine stimulation, and the initial light transient at lower lengths were not affected (Figures 2, 3, and 4). Decreases in tissue length from 1.2 to 1.0 $L_o$ also had no effect on aequorin light production (Figure 6). Furthermore, total light production per milligram of tissue (defined as $C_{max}$ per milligram wet weight at the beginning of the experiment) was independent of tissue length (3,200,000±460,000 counts/mg [n=10] at lengths less than or equal to 0.7 $L_o$ vs. 3,900,000±500,000 counts/mg [n=25]; $p=NS$). These findings suggest that attenuation of the light signal by changes in tissue thickness was not a major problem.

**FIGURE 7.** Graphs showing the change in length ($L$) (as a fraction of the optimal length for stress development [$L_o$]), log C/C$_{max}$ (intracellular [Ca$^{2+}$]), and active stress observed upon imposition of a 0.95-1.05 $L_o$, 1-Hz sinusoidal change in length at 10 minutes. The tissue was stimulated with 10 μM histamine at 20 minutes, and the oscillatory length change was turned off between 30 and 40 minutes. The [Ca$^{2+}$] data are shown in solid and dotted lines as described in Figure 2 with $n=4$, and the isometric control at 1.0 $L_o$ is replotted from Figure 3 and is shown in open squares. Representative length and stress data are from one tissue. Averaging of length and stress data was not performed because the different tissues were out of phase with each other. The apparent oscillations in the length and stress data at 0.05 Hz are an artifact of data sampling, but the minimum and maximum values of these oscillations reflect actual minima and maxima.

**FIGURE 8.** Graph showing the dependence of myosin phosphorylation on changes in log C/C$_{max}$ (intracellular [Ca$^{2+}$]) in isometric contractions preset at optimal lengths for stress development ($L_o$) of 0.7, 1.0, or 1.2 and stimulated with various doses of histamine. The data are from Figures 2, 3, 4, and 6, and the 1.0 $L_o$ data are replotted from Reference 3.

**Discussion**

This study confirms the result of Price et al$^{14-3}$ that shorter tissue lengths are associated with a decrease in agonist sensitivity (Figure 1) and suggests that the length dependence of agonist sensitivity is a general phenomenon. A similar decrease in agonist sensitivity of sustained values of myoplasmic [Ca$^{2+}$] at short tissue lengths was also observed (Figures 3 and 4). This result suggests that the mechanism of decreased agonist sensitivity is a length lowering of myoplasmic [Ca$^{2+}$], and not a decrease in the contractile apparatus sensitivity to [Ca$^{2+}$] (Figure 8). Shortening also reduced myoplasmic [Ca$^{2+}$] (Figure 5). This result differs from that of Gunst$^9$ in the electrically stimulated canine tracheals and may reflect differences between the tissue type or stimulus.

In contrast, agonist-induced isometric contractions at longer length (1.2 $L_o$) and during smaller oscillations in tissue length were not associated with significant alterations in the mobilization of intracellular [Ca$^{2+}$] by agonists (Figures 6 and 7).

Stretching the carotid media produced aequorin light transients that were not associated with proportional increases in myosin phosphorylation and stress development (Figures 1 and 6). The stretch-induced aequorin light signal without proportional activation can be interpreted in two ways. The first way is that stretching tissues may damage a few cells. Because aequorin has a steep dependence of light production on [Ca$^{2+}$], rupture of the cell membrane of a small proportion of cells (<1%) could induce the light signal observed in Figures 1 and 6. Bárány et al$^{10}$ reported that large stretches of the swine carotid (greater than 30%) increased myosin phosphorylation (measured 3 seconds after stretch) and that force redeveloped upon release. Stretches of this magnitude appear to induce stretch activation of...
their tissue and contract; however, such stretches impose extremely high passive stresses that may injure many cells and produce measurable phosphorylation and stress development.

Another more likely interpretation of our result is that stretch-induced [Ca\(^{2+}\)] transients may reflect generalized cell behavior. Contractile agonists such as histamine or phenylephrine exhibit the same [Ca\(^{2+}\)] dependence of phosphorylation. However, contractions dependent solely on extracellular Ca\(^{2+}\) influx are associated with a rightward shift (i.e., decreased sensitivity) in the [Ca\(^{2+}\)] dependence of phosphorylation. Examples of contractions totally dependent on extracellular Ca\(^{2+}\) include KCl depolarization and restoration of extracellular CaCl\(_2\) after depletion of both extracellular and intracellular Ca\(^{2+}\). The mechanism for the rightward shift is not known but may represent high [Ca\(^{2+}\)] in a subplasmalemmal compartment that is seen with Ca\(^{2+}\) entry. If stretch predominantly increased cell [Ca\(^{2+}\)] by increased Ca\(^{2+}\) influx, then large [Ca\(^{2+}\)] transients without proportional myosin phosphorylation or stress may be explained.

Some investigators, but not others, have reported that agonist stimulation was not associated with sustained elevation in quin 2- or fura-2-estimated myoplasmic [Ca\(^{2+}\)] in isolated cells. Based on the lack of sustained [Ca\(^{2+}\)] elevations, it was suggested that myoplasmic [Ca\(^{2+}\)] may not be responsible for sustained force maintenance in smooth muscle. However, dispersed cells are unloaded and can undergo extreme shortening at low levels of activation. Adhesion of cells to tissue culture substrates may prevent such extreme shortening; however, cells may already be quite shortened before attachment. The results of the current study suggest that short lengths and shortening are associated with attenuation of the sustained [Ca\(^{2+}\)] rise to some agonist concentrations. These findings suggest that utethered isolated cell preparations may exhibit shortening inactivation.

There are two major protocols used to estimate shortening velocity at zero load. Isotonic shortening velocity is determined by allowing contracted smooth muscle to shorten at various afterloads, measuring velocity at each afterload, and calculating unloaded velocity with Hill’s equation. This method typically involves shortening from 5% to 10% of L\(_{o}\), a range that did not demonstrably affect myoplasmic [Ca\(^{2+}\)]. In contrast, velocity of unloaded shortening is determined by step shortening of the contracted smooth muscle to various slack lengths and by measuring the time for force to start redeveloping. Typically, this method involves shortening of 20% to 30% of L\(_{o}\), a range that appears to reduce steady-state myoplasmic [Ca\(^{2+}\)]. The effect of shortening inactivation on the velocity of unloaded shortening is unknown.

In conclusion, short lengths and shortening were associated with a decrease in agonist sensitivity. The decrease in sensitivity was caused by decreased agonist-induced increases in myoplasmic [Ca\(^{2+}\)]. Agonist-induced increases in myoplasmic [Ca\(^{2+}\)] appear relatively insensitive to tissue lengths up to 1.2 L\(_{o}\) and smaller oscillatory changes.

References


**KEY WORDS** • aequorin • dose-response curves • histamine • length-tension relation • myosin phosphorylation • smooth muscle mechanics
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