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Longer muscle lengths recapitulate force suppression in swine carotid artery

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Abstract

Cyclic nucleotide can relax arterial smooth muscle without reductions in myosin regulatory light chain (MRLC) phosphorylation, a process termed force suppression. Smooth muscle contractile force also depends on tissue length. It is not known how tissue length affects force suppression. Swine carotid artery rings were equilibrated at various lengths (as a fraction of L_o , the optimal length for force development). They were then frozen during contractile activation with or without forskolin-induced relaxation. Frozen tissue homogenates were then analyzed for Ser¹⁹-MRLC phosphorylation and Ser¹⁶-heat shock protein 20 (HSP20) phosphorylation (HSP20 is the proposed mediator of force suppression). Higher values of MRLC phosphorylation were required to induce a histamine contraction at longer tissue lengths. At 1.4 L_o , the dependence of force on MRLC phosphorylation observed with histamine stimulation alone was shifted to the right, a response similar to that observed during force suppression at 1.0 L_o . The rightward shift in the dependence of force on MRLC phosphorylation seen with histamine stimulation alone at 1.4 L_o was not associated with increased HSP20 phosphorylation. Addition of forskolin to histamine-stimulated tissues at 1.4 L_o induced a relaxation associated with increased HSP20 phosphorylation and reduced MRLC phosphorylation, i.e., there was no additional force suppression. At shorter tissue lengths (0.6 L_o), the dependence of force on MRLC phosphorylation with histamine stimulation alone was steep, a response similar to that observed during normal contractile activation at 1.0 L_o . Addition of forskolin induced force suppression at 0.6 L_o . The sensitivity of swine carotid to the concentration of histamine was greater at longer tissue lengths compared with shorter tissue lengths, suggesting a physiological mechanism to restore optimal tissue length. These data suggest that longer tissue lengths induced a force suppression-like state that was 1) not additive with forskolin and 2) not associated with HSP20 phosphorylation. Further research is required to determine this length-dependent mechanism.

Keywords

heat shock protein-20; vascular smooth muscle

ARTERIAL SMOOTH MUSCLE CONTRACTION primarily involves stimulus-induced increases in Ser¹⁹-myosin regulatory light chain (MRLC) phosphorylation occurring via increases in myoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) (15). MRLC phosphorylation can also be increased by stimulus-induced inhibition of MRLC phosphatase (6,21). Increased Ser¹⁹-MRLC phosphorylation causes contraction. These processes are termed “activation.”

Relaxation can occur by two general mechanisms. First, “deactivation” is the reversal of activation involving dephosphorylation of MRLC by either a reduction in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) or an increase in MRLC phosphatase activity (6,8,14). Second, “force

suppression” is relaxation that occurs while MRLC phosphorylation levels remain elevated in the presence of excitatory stimuli (2,9,20). Phosphorylation of heat shock protein 20 (HSP20) on Ser¹⁶ (3,4,17,26) has been proposed to be the mediator of force suppression (10,17,20). HSP20 is also known as HspB6 (7).

The mechanism responsible for force suppression is unknown. One hypothesis is that Ser¹⁶ phosphorylation of HSP20 prevents cross bridges phosphorylated on MRLC from binding to the thin filament, a process that would reduce force despite high MRLC phosphorylation values (17). Specifically, this hypothesis proposes that cross-bridge binding is prevented by HSP20 binding to actin in a manner similar to troponin I [based on a sequence homology between HSP20 and the inhibitory region of troponin I (17)]. An alternative hypothesis is that Ser¹⁶-HSP20 phosphorylation could alter thin filament function by binding to and altering the function of actin filament binding proteins (5,24).

It is well known that changes in tissue length alter maximal force-generating capacity in smooth muscle (11). However, it is not known how tissue length affects force suppression. We hypothesized that 1) changes in length could per se induce a force suppression-like state and/or 2) changes in length could enhance or inhibit forskolin-induced force suppression. Therefore, we evaluated the dependence of force on MRLC phosphorylation in swine carotid artery at different lengths with and without forskolin treatment to induce relaxation.

MATERIALS AND METHODS

Tissues

Swine common carotid arteries were obtained from an abattoir and transported at 0°C in physiological salt solution. Physiological salt solution contained (in mM) 140 NaCl, 4.7 KCl, 5 MOPS; 1.2 Na₂HPO₄, 1.6 CaCl₂, 1.2 MgSO₄, 5.6 D-glucose; pH adjusted to 7.4 at 37°C. Dissection of medial rings, their being mounted, and the determination of the optimum length (L_0) for stress development at 37°C were performed as previously described (25). The intimal surface was mechanically rubbed to remove the endothelium.

Length changes

After L_0 was found, the length of each tissue was measured with a micrometer. Each chamber had a length adjuster with an attached 0.1-mm veneer that allowed releasing or stretching the tissue to the new length (L) as a fraction of the L_0 of the tissue. If the goal L/L_0 was <1.0, then the tissue was released to goal L/L_0 in one step and the force upon release was the passive force F_p . If the goal L/L_0 was >1.0, then the tissue was stretched in repeated steps until the tissue L/L_0 was ~0.1 L_0 above the goal L/L_0 , and then the tissue was released to the goal L/L_0 . The force upon release was the passive force F_p . A concentration of 109 mM K⁺ produces near-maximal and reliable contraction with consistent length tension curves, so forces were normalized to 109 mM K⁺ at both 1.0 L_0 and tissue length as described in the figure legends.

Measurement of HSP20 and MRLC phosphorylation

After pharmacological manipulation, tissues were frozen in an acetone dry ice slurry and homogenized in sodium didocyl sulfate as previously described (25). Full-strength, half-strength, and quarter-strength dilutions of samples were then separated on one-dimensional isoelectric focusing gels (ampholytes were a 50:50 mixture of pI 5–8 and pI 4–6.5 for HSP20 and a 50:50 mixture of pI 4.5–5.4 and pI 4.0–6.5 for MRLC), blotted, immunostained with our rabbit polyclonal anti-HSP20 antibody [1:5,000 on nitrocellulose in Tris-buffered saline with Tween-20 (19)] or rabbit polyclonal anti-MRLC antibody (1: 10,000 on polyvinylidene difluoride in 1% bovine serum albumin and 0.01% sodium azide, the antibody was a gift from Dr. Subah Packer), and detected with enhanced chemiluminescence. The dilutions ensured that

the enhanced chemiluminescence detection system was in the linear range (19). Immunoblots were imaged with a digital camera and quantitated with UnScanIt software.

RESULTS

Figure 1 shows how tissue length altered the steady-state dependence of force on MRLC phosphorylation. Tissues were first equilibrated at $1.0 L_0$ and contracted with 109 mM K^+ . Tissues were then shortened to 0.6 or 0.8 L_0 , unchanged at $1.0 L_0$, or lengthened to 1.2 or 1.4 L_0 . Sixty minutes later, the tissues were recontracted with 109 mM high K^+ at the new tissue lengths. Figure 1, *bottom*, shows the resulting classic length-tension relation induced by 109 mM K^+ : 1) increased F_p (triangles) at longer lengths, 2) highest 109 mM K^+ -induced F_a (circles) at $1.0 L_0$, and 3) lower 109 mM K^+ -induced F_a at lengths greater or less than $1.0 L_0$ (circles).

After washout of 109 mM K^+ , tissues were then contracted to 50% of the 109 mM K^+ force observed at each tissue length with either histamine or K^+ . There was some variability in the histamine and K^+ sensitivity between tissues, so the concentrations of histamine or K^+ were individually adjusted in each tissue to obtain 50% of 109 mM K^+ force previously observed in that tissue. Figure 1, *center*, shows the force measured at freezing, indicating that 50% of 109 mM K^+ force at tissue length was reliably obtained for each tissue. Tissues were then frozen, and MRLC phosphorylation was quantitated by isoelectric focusing (Fig. 1, *top*). The MRLC phosphorylation required to induce 50% of maximal force was significantly higher at longer tissue lengths ($P = 0.024$ by ANOVA and $P < 0.001$ by regression analysis: $\text{MRLC phosphorylation} = 0.156 + 0.114 \cdot L/L_0$, $r^2 = 0.21$). For both histamine (closed symbols) and K^+ (open symbols), higher MRLC phosphorylation values were required to induce 50% of maximal force at longer tissue lengths. These data suggest that longer tissue lengths altered the dependence of force on MRLC phosphorylation. Tissue lengths under $1.0 L_0$ did not significantly alter the dependence of force on MRLC phosphorylation.

In a separate experiment, we performed a more detailed evaluation of the effect of length on MRLC phosphorylation and force at 0.6 L_0 (Fig. 2, open squares) and 1.4 L_0 (closed circles). Tissues were stimulated with varying concentrations of histamine for 80 min and then frozen for measurement of MRLC phosphorylation. At 1.4 L_0 , contraction occurred at lower histamine concentrations than that observed at 0.6 L_0 (Fig. 2, bottom two panels). Histamine-induced MRLC phosphorylation levels were higher in the tissues at 1.4 L_0 compared with 0.6 L_0 (Fig. 2, *top*). At 100 μM histamine, force was lower and MRLC phosphorylation was higher at 1.4 L_0 compared with 0.6 L_0 , suggesting that there was less force for a given MRLC phosphorylation at 1.4 L_0 compared with 0.6 L_0 , i.e., there appeared to be force suppression at 1.4 L_0 .

Figure 3 shows the steady-state dependence of force on MRLC phosphorylation at 1.4 L_0 and 0.6 L_0 (data from Fig. 2). At 1.4 L_0 (closed circles), the dependence of force on MRLC phosphorylation was flatter and right shifted compared with the dependence of force on MRLC phosphorylation observed at 0.6 L_0 (open squares) or 1.0 L_0 (open star). A rightward shift in the dependence of force on MRLC phosphorylation fits the definition of force suppression, suggesting that histamine stimulation alone at 1.4 L_0 induced a state similar to force suppression.

We investigated whether the rightward shift in the dependence of force on MRLC phosphorylation observed with histamine stimulation alone at 1.4 L_0 was associated with increased Ser¹⁶ phosphorylation of HSP20. Unstimulated tissues and 1 μM histamine-stimulated tissues at 1.4 L_0 had low levels of Ser¹⁶-HSP20 phosphorylation compared with that observed in tissues stimulated with 10 μM histamine and relaxed with 0.4 μM forskolin

(Fig. 4). These data suggest that the force suppression-like state observed with histamine stimulation alone at $1.4 L_o$ was not caused by increases in Ser¹⁶-HSP20 phosphorylation.

In a third experiment, we evaluated the interaction between tissue length and cyclic nucleotide-induced force suppression (Fig. 5). Since longer lengths induced a rightward shift in the dependence of force on MRLC phosphorylation (similar to force suppression), we hypothesized that cyclic nucleotide-induced force suppression may not be observed at longer tissue lengths. Tissues were equilibrated at 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and $1.6 L_o$ as mentioned earlier and then contracted with 10 μ M histamine. Figure 5, *bottom*, shows the classic length-tension relation: 1) increased passive force (F_p , open triangles) at longer lengths (ANOVA $P < 0.001$), 2) highest histamine-induced active force (F_a H alone, open circles) at $1.0 L_o$, and 3) lower histamine-induced active force at lengths greater or less than $1.0 L_o$ (ANOVA $P < 0.001$).

Forskolin (0.2 μ M) was then added to all histamine-stimulated tissues. Forskolin induced a greater relaxation at 0.4, 0.6, 0.8, and $1.0 L_o$ than that observed at 1.2, 1.4, and $1.6 L_o$ lengths (Fig. 5, *bottom*, closed symbols, ANOVA $P < 0.001$). This greater relaxation at shorter lengths was best seen when force was normalized to the 109 mM K⁺-induced force observed at tissue length (Fig. 5, third panel, closed symbols, ANOVA $P < 0.001$). We tested whether difference in MRLC phosphorylation could explain the more pronounced relaxation at shorter lengths. Overall MRLC phosphorylation values significantly differed by ANOVA ($P = 0.027$); however, pair-wise comparison did not distinguish which values differed (Fig. 5, second panel). There was no difference in Ser¹⁶-HSP20 phosphorylation at different lengths (Fig. 5, *top*, ANOVA $P = 0.42$). These data suggest that forskolin-induced relaxation/force suppression is less apparent at longer tissue lengths. It is possible that the more pronounced relaxation at 0.4 and $0.6 L_o$ may be caused by lower MRLC-phosphorylation values.

A more detailed dependence of force on MRLC phosphorylation during forskolin-induced relaxation at 0.6 and $1.4 L_o$ is shown in Fig. 6 (this experiment was performed as part of the experiment performed in Figs. 2 and 3). At $1.4 L_o$, the addition of 0.2, 0.4, or 1.0 μ M forskolin to a 10 μ M histamine contraction induced a dependence of force on MRLC phosphorylation (Fig. 6, *top*, closed circles) similar to the flattened dependence of force on MRLC phosphorylation observed with histamine alone (Fig. 6, *top*, open circles). These data suggest that the relaxation observed with forskolin at $1.4 L_o$ was secondary to the reduction in MRLC phosphorylation, i.e., it was deactivation and there was no forskolin-induced force suppression. In contrast, at $0.6 L_o$, the addition of 0.1, 0.2, or 0.4 μ M forskolin to a 10 μ M histamine contraction induced a rightward shift in the dependence of force on MRLC phosphorylation (Fig. 6, *bottom*, closed squares) compared with the steeper dependence of force on MRLC phosphorylation observed with histamine alone (Fig. 6, *bottom*, open squares). These data suggest that the forskolin-induced relaxation at $0.6 L_o$ was caused by force suppression rather than by a reduction in MRLC phosphorylation.

DISCUSSION

Longer lengths recapitulate force suppression

The dependence of force on MRLC phosphorylation depended on tissue length. Tissues at the optimal length for force generation ($1.0 L_o$) and at shorter lengths had a steep dependence of force on MRLC phosphorylation (Fig. 3, solid line) that was also demonstrated by a lower MRLC phosphorylation value required to induce 50% of maximal force (Fig. 1). Tissues at lengths greater than L_o had a flatter dependence of force on MRLC phosphorylation (Fig. 3, dashed line) that was also manifested as significantly higher MRLC phosphorylation values required to induce 50% of maximal force (Fig. 1). This effect on longer tissue lengths did not

depend on the type of activation given that similar results were seen with histamine and high $[K^+]$ (Fig. 1).

Force suppression was originally described as a rightward shift in the dependence of force on MRLC phosphorylation that occurred with cyclic nucleotide-induced relaxation of swine carotid tissues at $1.0 L_0$ (16,17). Our data suggest that force suppression also occurs with forskolin-induced relaxation at shorter tissue lengths ($0.6 L_0$, Fig. 6, *bottom*). However, further force suppression was not observed with forskolin-induced relaxation at longer tissue lengths ($1.4 L_0$, Fig. 6, *top*). These data have several implications. First, longer lengths appear to induce a force suppression-like state, i.e., a dependence of force on MRLC phosphorylation similar to that observed during cyclic nucleotide-induced force suppression (Fig. 6, compare histamine alone at $1.4 L_0$ with histamine plus forskolin at $0.6 L_0$). Second, forskolin-induced force suppression was not additive with the force suppression-like state observed at $1.4 L_0$.

Does this suggest that cyclic nucleotide-induced force suppression shares a common mechanism with the force suppression-like state observed at $1.4 L_0$? HSP20 phosphorylation is hypothesized to mediate cyclic nucleotide-induced force suppression (17). However, increased levels of HSP20 phosphorylation were not found in tissues stimulated with histamine alone at longer tissue lengths ($1.4 L_0$, Fig. 4). This suggests that the flattening of the dependence of force on MRLC phosphorylation at longer tissue lengths was not mediated by Ser¹⁶-HSP20 phosphorylation.

The mechanism for this length effect is unknown. However, there is a recent body of evidence suggesting that activation-induced assembly and/or organization of thin filaments is required for airway smooth muscle contraction. Based on a strategy of inhibitors, antisense, siRNA, and mutant proteins, Gunst and colleagues (23) showed that interference with several tracheal smooth muscle proteins in the actin polymerization pathway interfered with actin polymerization and contraction without altering MRLC phosphorylation values 5 min after activation. Their proposed mechanism for actin polymerization in tracheal smooth muscle (23) is for contractile agonist activation to activate FAK, inducing paxillin phosphorylation, causing CrkII coupling and formation of the CrkII/Cdc42/N-WASp complex, causing Cdc42 activation, N-WASp activation, activation of the Arp2/3 complex, and actin polymerization. These data suggest that a second system beyond MRLC phosphorylation may be either permissive for or involved in regulating force in tracheal smooth muscle. We found an increase in actin polymerization upon histamine stimulation of swine carotid artery (10), suggesting that a similar mechanism is operating in arterial smooth muscle. Potentially, a length dependence of actin filament polymerization could explain some of the length-dependent changes in the dependence of force on MRLC phosphorylation observed in this study.

The literature is limited on how changes in length affect the dependence of force on MRLC phosphorylation. Almost all studies involve the effect of tissue length on the response to maximal contractile stimulation; therefore, the dependence of force on MRLC phosphorylation cannot be determined. The only exception was a study of bovine trachealis in which shorter lengths did not alter the dependence of force on MRLC phosphorylation (27). It should be noted that airway smooth muscle, such as trachealis, differs significantly from arterial smooth muscle in that the trachealis has a very plastic length-force relation (1). Our laboratory (18) previously studied the swine carotid artery stimulated with various concentrations of histamine at $0.7 L_0$. In the publication, we did not plot the dependence of force on MRLC phosphorylation. An analysis performed when writing this paper showed no change in the dependence of force on MRLC phosphorylation at $0.7 L_0$, similar to the current results at $0.6 L_0$. Two studies in maximally activated arterial smooth muscle showed that longer tissue lengths were associated with higher MRLC phosphorylation values (22,25), results similar to those in trachealis (27).

Longer lengths are associated with increased sensitivity to agonist concentration

The current experiments also showed that swine carotid has different sensitivity to histamine-induced contraction at different tissue lengths. Specifically, tissues at long lengths were more sensitive to the concentration of histamine than tissues at shorter lengths (Fig. 2). These results confirm prior results in the canine anterior tibial artery (12,13). Specifically, Price et al. (12, 13) showed lower norepinephrine sensitivity at shorter lengths and increased sensitivity at longer lengths. Previously, our laboratory reported a reduced sensitivity to histamine at shorter lengths (18). A mechanism for this response is unknown. Such a mechanism does have clear physiological advantages to maintain tissue lengths near optimal length during agonist stimulation ($1.0 L_0$). If tissues are stretched to longer lengths, then the increased sensitivity to agonists would result in more shortening to optimal length. Conversely, if tissues are shortened to shorter lengths, then the reduced sensitivity to agonists would allow lengthening to optimal length.

The difference in histamine sensitivity at different lengths also shows the need to measure full plots of the dependence of force on MRLC phosphorylation, e.g., Figs. 3 and 6, because evaluation at any given single concentration of histamine would not clearly delineate the physiology.

In conclusion, these data suggest that long tissue lengths alter the dependence of force on MRLC phosphorylation. Specifically, longer tissue lengths appear to induce a force suppression-like state that occurs without increases in Ser¹⁶-HSP20 phosphorylation. Further research is required to evaluate the mechanism for this effect.

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Fig. 1.

Effect of tissue length on the steady-state dependence of force on myosin regulatory light chain (MRLC) phosphorylation in swine carotid artery rings. Rings were first contracted with 109 mM K^+ at 1.0 optimum length (L_0). After washout of high K^+ , length was adjusted as shown on the abscissa, and the tissues recontracted with 109 mM K^+ at the new length. *Bottom*, resulting high K^+ length-tension curve with passive forces (F_p , triangles) zero at lower lengths and increasing at lengths above 1.0 L_0 . 109 mM K^+ -induced active force (F_a , circles) was maximal at 1.0 L_0 and decreased at both shorter and longer lengths (closed circles represent those tissues that were subsequently stimulated with histamine and open circles those subsequently depolarized with high K^+). After washout of high K^+ , tissues were then activated to ~50% of 109 mM K^+ force observed at that tissue length with various concentrations of histamine (closed circles) or K^+ (open circles) as shown in the central panel (time at freezing was variable but always was >30 min). *Top*, tissues were then frozen and the MRLC phosphorylation measured. This level of MRLC phosphorylation (presented as mol P_i /mol protein) is that required to induce 50% of maximal at that tissue length. Data are presented as means $\pm$ SE, and symbols conceal some small error bars ($n = 4 - 8$). The data show that higher MRLC phosphorylation was required to induce 50% force in tissues stretched to longer lengths.

Fig. 2.

Histamine dose response at 1.4 L_0 (closed circles) and 0.6 L_0 (open squares) in swine carotid artery rings. Rings were first contracted with 109 mM K^+ at 1.0 L_0 , and then length was adjusted to 1.4 or 0.6 L_0 (this experiment was a separate set of carotid tissues than those examined in Fig. 1). At the new length, tissues were recontracted with 109 mM K^+ , relaxed, and then contracted for 80 min with varying concentrations of histamine as shown on the abscissa.

Bottom, histamine-induced active force as a percentage of the K^+ contraction at 1.0 L_0 .

Center, histamine-induced active force as a percentage of the K^+ contraction at the actual length (1.4 or 0.6 L_0).

Top, measured steady-state MRLC phosphorylation. Data are presented as means $\pm$ SE and symbols conceal some small error bars ($n = 4-7$). The data show that higher concentrations of histamine were required to induce contraction at shorter lengths.

Fig. 3.

The steady-state dependence of force (as a percentage of the 109 mM K⁺ contraction at tissue length) on MRLC phosphorylation (as a fraction of total MRLC) in swine carotid artery rings at 1.4 L₀ (closed circles) or 0.6 L₀ (open squares). Data are replotted from Fig. 2. Open star, data at 1.0 L₀ from Fig. 1. Data are presented as means ± SE and symbols conceal some small error bars (*n* = 4–7). Outside the intersection at low force, the 95% confidence lines of each regression did not include the other regression line (not shown for clarity), indicating that the two regressions differed significantly. The data show a rightward shift in the dependence of force on MRLC phosphorylation at 1.4 L₀ compared with that observed at 0.6 or 1.0 L₀.

Fig. 4.

Ser¹⁶-heat shock protein (HSP)20 phosphorylation in swine carotid artery rings at 1.4 L_0 . Rings were unstimulated, stimulated with 1 μ M histamine for 80 min, or stimulated with 10 μ M histamine for 20 min and relaxed by addition of 0.4 μ M forskolin for an additional 60 min (this experiment is a subset of the experiment shown in Figs. 2 and 6). Data are presented as means $\pm$ SE and symbols conceal some small error bars ($n = 4$). The data show significant increases in Ser¹⁶-HSP20 phosphorylation in only the forskolin-treated rings.

Fig. 5.

Effect of tissue length on forskolin-induced relaxation in swine carotid artery rings. Rings were first contracted with 109 mM K⁺ at 1.0 L₀, and then length was adjusted as shown on the abscissa (this experiment was a separate set of carotid tissues than those examined in Figs. 1–4). Tissues were then recontracted with 109 mM K⁺ at the new length, and the high K⁺ was washed out. Tissues were then contracted with 10 μM histamine for 20 min, and the bottom panel shows the resulting histamine-induced length-tension curve with passive force (F_p, open triangles) and active forces (F_a, open circles) as a percentage of the K⁺ contraction at 1.0 L₀. Forskolin (0.2 μM, 60 min) was then added to the histamine-contracted tissues, and the tissues were frozen after 60 min in histamine and forskolin. Circles, steady-state forskolin and histamine response. *Bottom panel*, force as a percentage of the 1.0 L₀ K⁺ force; *third panel*, force at tissue length; *second panel*, MRLC phosphorylation; *top panel*, Ser¹⁹-HSP20 phosphorylation. Left, unstimulated tissues (labeled control). Data are presented as means ± SE and symbols conceal some small error bars (n = 4 – 8). Forskolin relaxed shorter tissues better than longer tissues despite similar MRLC and Ser¹⁶-HSP20 phosphorylation values (at 0.4 and 0.6 L₀, MRLC phosphorylation appeared to be slightly lower but did not make statistical significance).

Fig. 6.

Steady-state dependence of force (as a percentage of the 109 mM K^+ contraction at tissue length) on MRLC phosphorylation in histamine-stimulated swine carotid artery rings with or without forskolin at 1.4 L_o (top) and at 0.6 L_o (bottom). The histamine alone data (open symbols) are from Fig. 2. At 1.4 L_o , tissues were stimulated with 10 μ M histamine for 20 min and then relaxed by addition of 0.2, 0.4, or 1.0 μ M forskolin for 60 min (top, closed circles), demonstrating no difference in the dependence of force on MRLC phosphorylation with the addition of forskolin. At 0.6 L_o , tissues were stimulated with 10 μ M histamine for 20 min and then relaxed by addition of 0.1, 0.2, or 0.4 μ M forskolin for 60 min (bottom, closed squares), demonstrating a rightward shift in the dependence of force on MRLC phosphorylation with the addition of forskolin. Data are presented as means $\pm$ SE and symbols conceal some small error bars ($n = 4-7$, the forskolin-treated tissues were performed at the same time as the histamine alone data presented in Fig. 2). The data show force suppression (a rightward shift

in the dependence of force on MRLC phosphorylation) in the tissues at $0.6 L_o$ but not at $1.4 L_o$.