Force–Velocity Relation and Myosin Light Chain Phosphorylation in Bovine Coronary Arterial Smooth Muscle

Wanda C. Miller-Hance and Kristine E. Kamm

We have investigated in bovine left ventricular coronary arteries the relation between the biochemical regulatory event of myosin light chain phosphorylation and the mechanical events of isometric stress and isotonic shortening, under conditions of stimulation by depolarization (65 mM KCl) or receptor occupancy (2 μM 5-hydroxytryptamine [5-HT]). At rest, levels of light chain phosphorylation were 0.07±0.01 mol phosphate/mol light chain. Maximal values were significantly different for KCl (0.42±0.02 mol phosphate/mol light chain at 1 minute) and 5-HT stimulation (0.58±0.01 mol phosphate/mol light chain at 30 seconds). Increases in light chain phosphorylation preceded isometric stress development, and values remained elevated at ~0.35 mol phosphate/mol light chain for up to 2 hours with both KCl and 5-HT. The sites of phosphorylation were identical for KCl and 5-HT at 2 hours. Maximal stresses for each stimulus were also maintained for 2 hours. Values of maximum velocity of shortening (V₀ in muscle lengths [ML]/sec), obtained from the force–velocity relation, did not change significantly between 1 minute and 2 hours with KCl (0.070±0.008 ML/sec at 1 minute and 0.056±0.007 ML/sec at 2 hours, p>0.2). However, during 5-HT stimulation, V₀ declined significantly (0.053±0.006 ML/sec at 1 minute and 0.032±0.003 ML/sec at 2 hours, p<0.025). The relation between V₀ and light chain phosphorylation was different for KCl and 5-HT, indicating that factors in addition to myosin light chain phosphorylation may modulate smooth muscle shortening velocity. (Circulation Research 1991;69:1207–1214)

Smooth muscle contraction is initiated by phosphorylation of myosin at a specific residue on the 20,000-d light chain subunit by Ca²⁺- and calmodulin-dependent myosin light chain kinase.¹⁻⁴ Phosphorylation of the light chain results in stimulation of actin-activated MgATPase activity of smooth muscle myosin, which in the muscle fiber is manifest as a cyclic interaction of phosphorylated crossbridges with thin filaments, resulting in development of force. The scheme for activation proposes that changes in cytoplasmic Ca²⁺ concentrations are responsible for the initiation and maintenance of smooth muscle contraction, and studies in a variety of intact and functionally skinned smooth muscle preparations have demonstrated the dependence of force generation on Ca²⁺ concentration and myosin phosphorylation, confirming the important role for phosphorylation in contraction.

Investigations on intact muscle have, however, also revealed variations in the relation between mechanical properties of contraction and levels of both Ca²⁺ and myosin phosphorylation among various types of smooth muscle.¹⁻⁵ Some smooth muscles, for example, exhibit a biphasic pattern of activation. An initial transient phase is associated with increasing Ca²⁺ concentrations, rapid increases in myosin phosphorylation, and concomitant increases in shortening rates (crossbridge cycling rates), followed by the development of active stress. During a second sustained phase of the contraction, stress is maintained in the presence of falling Ca²⁺ concentrations, elevated but low levels of myosin phosphorylation, and slow shortening rates. The latter set of conditions defines functionally the "latch" state, in which force is maintained economically as crossbridge cycling rates (hence ATP consumption) are reduced.⁴ Hai and Murphy⁴,⁵ have developed a four-state kinetic model of latch, in which force is maintained by two populations of crossbridges: phosphorylated rapidly cycling bridges and dephosphorylated attached non-cycling bridges (latchbridges). Application of A.F. Huxley's formulation of crossbridge cycling to the four-state model predicts a linear dependence of

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maximal shortening velocity on myosin light chain phosphorylation. Such a linear dependence has been observed in a number of smooth muscle preparations, including the bovine trachealis. The present study was undertaken to investigate the contractile properties of bovine left ventricular coronary arteries to assess the relation between the mechanical events of isometric stress and isotonic shortening and the biochemical regulatory event of myosin light chain phosphorylation under different conditions of activation. We hypothesized that stimulation by depolarization or receptor occupancy would result in different patterns of activation that would reveal aspects of vascular smooth muscle function that may be unique to the coronary artery. In consideration of the physiological implication of the twitch state and the possible relation to arterial vasospasm, the aim of our investigation was also to determine whether coronary arterial smooth muscle exhibits this behavior, as has been demonstrated by another well-characterized smooth muscle of the same animal species, the bovine trachealis. Our findings show that, unlike the trachealis, coronary arterial smooth muscle maintains intermediate levels of myosin light chain phosphorylation during prolonged force maintenance in response to an agonist or depolarization. Additionally, the present results show that in bovine coronary arterial muscle the maximal velocity of shortening (V_o) can vary over a nearly twofold range at a given level of light chain phosphorylation. These results suggest that factors in addition to myosin light chain phosphorylation may modify crossbridge cycling rates in smooth muscle.

Materials and Methods

Drugs and Solutions

The physiological salt solution (PSS) used for preparation of tissues and as a bathing medium was of the following composition (mM): NaCl 120.5, KCl 4.8, MgSO_4_ 1.2, NaH_2PO_4_ 1.2, NaHCO_3_ 20.4, CaCl_2_ 1.6, d-glucose 10.0, and pyruvate 1.0. For solutions of high potassium concentration (KCl), KCl was substituted for NaCl in equimolar amounts. 5-Hydroxytryptamine (5-HT) was purchased from Sigma Chemical Co., St. Louis, Mo. The drug was prepared as a concentrated stock solution and diluted appropriately in the bathing medium.

Tissue Preparation

Bovine hearts were collected at a local abattoir immediately after slaughter and transported to the laboratory on ice. A segment of the left anterior descending coronary artery, ~50–80 mm from the left main coronary artery bifurcation, was isolated in all specimens by freeing the vessel from adjacent adipose, cardiac, and loose connective tissue. Approximately the same segment was isolated from all specimens to avoid possible complicating factors in data interpretation, such as regional heterogeneity of pharmacological responses as reported in various vascular preparations. Several rings of uniform width (1.8 mm) were cut perpendicular to the longitudinal axis of the vessel because of the predominant transverse orientation of smooth muscle cells in this tissue. The PSS, aerated with a 95% O_2–5% CO_2 gas mixture (pH 7.4 at 37°C), was used to keep the tissue moist. After exposing the vascular lumen by opening the rings, the intimal surface was gently swabbed with a cotton tip applicator to remove the endothelium.

Mechanical Measurements

Isometric force. The vascular strips, which were mounted vertically and immersed in 25-ml jacketed tissue chambers, were bathed in gassed PSS maintained at 37°C. Mounting attachments maintained the rectangular geometry of these vascular muscle strips. Passive and active length–force relations in bovine coronary arterial strips were determined in preliminary experiments according to the method described by Herlihy and Murphy. At the optimal length for force generation (L_o), the ratio between passive and active force was ~0.20. The establishment of L_o was important for comparison of pharmacological responses among strips. In subsequent experiments, arterial tissues were passively stretched to a length obtained with two applications of 50 mN force, and after a 2-hour period of stress relaxation, the muscles were adjusted to L_o. Average force generated by these muscle strips in response to high potassium PSS was 110 mN. Strips were rapidly frozen after stimulation with 65 mM KCl or 5-HT. Frozen tissues were stored at −60°C. The magnitudes of the contractile responses at the time of freezing are expressed as a fraction of the force obtained during a maximal precontraction with a given stimulus. Active stress (N/m²) was determined in strips not frozen for biochemical analysis, after length and wet weight of the muscles were recorded and values of force were normalized for tissue cross-sectional area. Isotonic shortening velocity. Force–velocity relations were determined with an electronic servo control system under computer control. Experimental protocols and data acquisition were under the control of a North Star Horizon II computer that was interfaced with the servo electronics (Cambridge Technology, Cambridge, Mass.). Coronary strips were hung from the lever arm and inserted into a Plexiglas base that was attached to a stainless-steel rod mounted on a calibrated drive. Both position and force were monitored from the servo electronics with a recorder (model 7745A, Hewlett-Packard Co., Palo Alto, Calif.) and simultaneously digitized and stored for further analysis. For isotonic quick release experiments, a digital-to-analog converter was used to set a selected voltage at the force input of the servo electronics. This determined the level of the isotonic afterload against which the muscle shortened. A basic program was used to construct release protocols, which were executed by programs written in 80 assembly language.
**Table 1. Force-Velocity Parameters for Bovine Coronary Artery**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KCl 1 min</th>
<th>KCl 2 hr</th>
<th>5-HT 1 min</th>
<th>5-HT 2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (10^6 N/m²)</td>
<td>1.09±0.06</td>
<td>1.44±0.10*</td>
<td>0.94±0.07</td>
<td>1.01±0.10*</td>
</tr>
<tr>
<td>V₀ (ML/sec)</td>
<td>0.070±0.008</td>
<td>0.056±0.007</td>
<td>0.053±0.006</td>
<td>0.032±0.003*</td>
</tr>
<tr>
<td>a/F</td>
<td>0.37±0.06</td>
<td>0.27±0.03</td>
<td>0.51±0.11</td>
<td>0.45±0.09</td>
</tr>
<tr>
<td>b (ML/sec)</td>
<td>0.023±0.002</td>
<td>0.015±0.001*</td>
<td>0.024±0.003</td>
<td>0.014±0.002*</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. 5-HT, 5-hydroxytryptamine; F*, isometric force development at the time of release; V₀, maximum velocity of shortening; a and b, negative values of force and velocity asymptotes, respectively, of the hyperbolic Hill equation; n, number of animals. Parameters were derived from individual force (F)–velocity (V) curves calculated from linear regression on data transformed to the linear form of the hyperbolic Hill equation: (F+a)(V+b)=(F+a)b. No 1-minute values in 5-HT were significantly different from 1-minute values in KCl.

*p<0.05 vs. 1-minute value for same stimulus; †p<0.05 vs. 2-hour value for KCl.

**Determination of Myosin Phosphorylation**

Phosphorylation of the light chain in arterial smooth muscle samples was determined as previously described for the study of tracheal smooth muscle, except for minor modifications required for coronary vessels.13,14 Frozen coronary arterial tissues were homogenized in 60 vol (vol/wt) extraction buffer that included 100 mM sodium pyrophosphate, 50 mM sodium fluoride, 5 mM EGTA, 10% glycerol, 3 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM leupeptin, and 100 units/ml aprotinin (final pH 8.8). After homogenization at 0°C, potassium iodide and Triton X-100 were added for a final concentration of 0.55 M and 1%, respectively, in the tissue homogenates. After incubation at 0°C for 20 minutes, the smooth muscle extracts were centrifuged at 7,000g for 15 minutes. Protein in the supernatant fraction was precipitated by the addition of 100% trichloroacetic acid at 0°C, for a final concentration of 10%. After centrifugation, the pellets were extracted with diethyl ether and suspended in 30 vol (vol/wt) of a buffer containing 8 M urea, 10 mM dithiothreitol, 20 mM Tris base, 23 mM glycine, and 0.004% bromophenol blue (pH 8.6). The glyceraldehyde-3-phosphate dehydrogenase, as well as the electrophoretic transfer of proteins onto nitrocellulose paper, and immunoblotting of the nonphosphorylated and phosphorylated forms of the light chain were performed as previously described.14 Quantitation of the extent of myosin phosphorylation was accomplished by laser densitometry of nonphosphorylated and phosphorylated light chain forms.

**Determination of Sites of Myosin Light Chain Phosphorylation**

Strips of coronary artery were prepared as described previously for ⁴⁵Ca labeling of trachealis muscle.15 Strips were mounted isometrically on stainless-steel wire holders and incubated for 4 hours at 37°C in PSS containing 125 μCi/ml [³²P]NH₄H₂PO₄. After exposure to experimental solutions, strips were frozen by submersion in dichlorodifluoromethane chilled in liquid nitrogen and then stored at -60°C. Two-dimensional peptide mapping of ⁴⁵Ca-labeled light chain was performed as described.15 Myosin was immunoprecipitated from homogenates of labeled strips. Eluted protein was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and gel areas containing myosin light chain (identified by Coomassie blue staining) were excised. Gel slices were incubated in 1 ml of 25 mM NH₄HCO₃ (pH 8.4) and 100 μg trypsin overnight at 37°C. Sample digests were dried, resuspended in electrophoresis buffer, and subjected to electrophoresis and chromatography.15 Autoradiography was performed to locate ²⁵Ca phosphopeptides.

**Statistical Analysis**

All results of averaged data are expressed as mean±SEM. Comparison of mean values was by Student’s unpaired t test. Statistical significance between values was assumed at p<0.05.

**Results**

**Isometric Force Responses to KCl and 5-HT**

Studies of the mechanical properties and myosin phosphorylation in coronary arterial smooth muscle were conducted in strips stimulated with KCl and 5-HT. Among a number of contractile agents tested (carbachol, norepinephrine, phenylephrine, methoxamine, and histamine), including electrical stimulation, these proved to yield the highest stresses.

The relation between the concentrations of KCl or 5-HT and the magnitude of isometric force was determined. The addition of either agent to the tissue bath evoked concentration-dependent increases in active force, with concentrations of 13 mM and 0.3 μM required for half-maximal response for KCl and 5-HT, respectively. Concentrations that produced between 90% and 98% of maximal force for each agent (65 mM for KCl and 2 μM for 5-HT) were used in subsequent experiments. KCl yielded greater maximal stresses than 5-HT at 2 hours of stimulation (Table 1).

The relative contribution of extracellular calcium to the contractile response for each stimulus was determined by removing Ca²⁺ from the PSS. In control experiments, strips were contracted in regular PSS in the presence of each agent until maximal isometric response was attained. After relaxing the
tissues, the strips were incubated for a period of 10 minutes in Ca\(^{2+}\)-free PSS containing 2 mM EGTA. On reexposure of the muscles to KCl, a negligible increase in active force was observed, with maximum values reaching 2±0.5% of pretreatment levels. The 5-HT stimulated strips, however, exhibited a transient but significant increase in active force, with maximum values of 30±2% control levels. The transient peaked at 1.4±0.1 minutes after the addition of the agonist, and force declined to levels of ~20±2% of maximum after 30 minutes of stimulation. Replacement of Ca\(^{2+}\) in the PSS restored the magnitude of the isometric response elicited by each agent to control values. These findings suggest that 5-HT mobilizes Ca\(^{2+}\) from intracellular sites. Although the presence of Ca\(^{2+}\) in the extracellular compartment contributes partially to both the phasic and the tonic components of the 5-HT response, it is absolutely required for contractile response to KCl.

Myosin Phosphorylation

Study of the time course of myosin light chain phosphorylation in coronary arterial smooth muscle (Figure 1) revealed low resting levels of 0.07±0.01 mol phosphate/mol light chain in unstimulated tissues. Phosphorylation increased rapidly to a maximum value within 15 seconds of KCl stimulation and 30 seconds of 5-HT application. However, peak values for phosphate content of the light chain were significantly different under the two conditions of activation, with levels of 0.39±0.02 mol phosphate/mol light chain for KCl as opposed to 0.58±0.01 mol phosphate/mol light chain for 5-HT (p<0.025). Initial isometric force responses of these muscles followed a similar time course in response to KCl and 5-HT (Figure 1). Later, however, KCl-stimulated muscles demonstrated a slower rate of isometric force development. Rises to maximum force in strips stimulated with 5-HT required only ~3 minutes, whereas tissues contracted with KCl did not reach maximum values until between 10 and 20 minutes of stimulation. Increases in myosin phosphorylation preceded changes in isometric force, and although there was a decrease with time in the extent of phosphorylation among 5-HT-contracted vascular tissues, levels considerably above basal values were maintained for prolonged periods of stimulation in response to both KCl and 5-HT. At 2 hours of stimulation, phosphorylation values were similar under the two conditions of activation.

Isotonic Shortening Velocities

Force–velocity measurements were performed by the method of quick release to a fixed afterload. Individual releases to five or six loads were performed on consecutive contractions to construct the force–velocity relations at 1 minute. Quick releases were performed on a single contraction for measurements made after 2 hours of stimulation. The order of treatment (1 minute or 2 hours with KCl or 5-HT) was varied randomly among strips. The order of applica-

![Graph showing effect of KCl and 5-hydroxytryptamine on myosin light chain phosphorylation and force.](image)

**FIGURE 1.** Graphs showing effect of KCl and 5-hydroxytryptamine on myosin light chain phosphorylation and force. Top panel: Bovine coronary arterial strips frozen at the indicated times as controls (x) and after the addition of 65 mM KCl (●) or 2 μM 5-HT (○). Quantitation of light chain phosphorylation was performed as described in “Materials and Methods.” Bottom panel: Temporal force responses of muscles frozen for measurement of light chain phosphorylation. Force (F) is expressed as a fraction of maximal active force (F\(_a\)) for each stimulus. Values represent the mean±SEM for four to nine samples.

tion had no effect on the force–velocity relation for a given treatment. Afterloads were also applied in random order. The previous application of any load had no effect on the response to subsequent releases, as determined by the degree of fit of the force–velocity relations (r\(^2\)=0.95±0.007, n=18) to a hyperbolic function. The length response consisted of a rapid elastic recoil followed by a phase of slow shortening that could be described by a single exponential. Measurements were made on muscles whose length was adjusted to 0.9 L\(_{oo}\), where passive forces were <10% maximal force, to minimize the load transferred onto the contractile element during shortening. Shortening velocities were calculated by a least-squares fit of the position data to an exponential relation. Position data between 150 and 500 msec were used, and shortening was normalized to muscle length. Velocity was calculated as the slope of the curve when extrapolated to time zero. The parameters of the force–
velocity relation were calculated from a linear transform of the hyperbolic Hill equation. Individual values are plotted in Figure 2 along with curves derived from the average values shown in Table 1. Values of \( V_0 \) did not change between 1 minute and 2 hours with KCl, whereas during 5-HT stimulation, \( V_0 \) declined significantly. A second difference in responses of the coronary arterial muscle relates to the steady-state stress and \( V_0 \), which was significantly greater in KCl than in 5-HT (Figure 1); values of light chain phosphorylation did not differ between the two conditions (Figure 1).

Sites of Phosphorylation

Comparison of the phosphorylation–velocity relations for the two agonists revealed that in the presence of KCl higher values of \( V_0 \) were obtained for a given level of light chain phosphorylation (Figure 3). We tested the hypothesis that the velocity of shortening may be modulated by phosphorylation of the light chain at sites other than serine 19. Peptide maps of light chain from \( ^{32} \)P-labeled coronary arteries, however, showed identical patterns of phosphopeptides with KCl and 5-HT stimulation (Figure 4). The majority (80–90%) of the \( ^{32} \)P was in the serine 19 site phosphorylated by myosin light chain kinase; however, under both conditions \( ^{32} \)P was also seen in phosphopeptides containing threonine 18 (<5%) and serine 1 or 2 (5–10%).

Discussion

An understanding of the relation between mechanical activity and the biochemical processes associated with activation is important in the study of regulation of smooth muscle contraction. In particular, efforts to evaluate these properties in the bovine coronary artery not only represent an attempt to study normal arterial smooth muscle function for comparison with the well-characterized bovine airway smooth muscle model but may also be of relevance to future investigations of pathological states related to altered coronary blood flow.

Length–force relations were established for the bovine left ventricular coronary artery. The curves generated were similar to those obtained for other arterial smooth muscles. However, values of stress are markedly greater than those previously reported for other coronary artery preparations, in-

![Figure 2. Force–velocity relations for bovine coronary arterial strips. Velocity in muscle lengths (ML) per second is shown as a function of isotonic load after quick release (F) expressed relative to isometric force development at the time of release (F'). Individual observations are plotted along with average curves for KCl (triangles, left panel) and 5-hydroxytryptamine (circles, right panel) stimulation for 1 minute (open symbols, dashed lines) or 2 hours (closed symbols, solid lines). Number of animals are the same as shown in Table 1.](image)

![Figure 3. Graph showing relation between myosin light chain phosphorylation and maximal velocity of shortening (\( V_0 \)). ML, muscle length; 5-HT, 5-hydroxytryptamine. Data are derived from Table 1 and Figure 1. Closed square represents myosin light chain phosphorylation in muscles at rest. Triangles indicate KCl stimulation and circles indicate 5-HT stimulation for 1 minute (open symbols) or 2 hours (closed symbols). It is assumed that relaxed muscle will not shorten; therefore, \( V_0 \) is zero.](image)
cluding bovine.\textsuperscript{17,18} This difference may be attributable to the variability in experimental conditions from one laboratory to another, including factors such as modes of activation, isometric measurements at muscle lengths other than \(L_0\), differences in the absolute amounts or the proportions of contractile proteins in a given tissue, or cellular damage during the tissue preparation.\textsuperscript{19} Comparatively, studies in other smooth muscles (e.g., bovine trachealis and porcine carotid artery) have demonstrated higher values of active stress than those observed herein for the coronary artery.\textsuperscript{7,19}

In KCl-contracted bovine coronary artery, the relative rate of isometric force generation (normalized to steady-state force) was slower when compared with that observed in 5-HT-stimulated muscles, suggesting that these two agents may induce activation of the contractile system through different pathways. The maximal extent of phosphate incorporation into the light chain was also different between the two agents. Starting from low levels at rest, 5-HT produced higher peak values of net light chain phosphorylation, as compared with levels observed in KCl. Therefore, in the coronary artery, as demonstrated for the swine carotid artery, relative force develops slowly in the case where no initial transient in myosin light chain phosphorylation occurs.\textsuperscript{20} The hypothesis that the effects of these agents are mediated through the mobilization of different pools of \(\text{Ca}^{2+}\) is supported by our results showing that removal of extracellular sources of activator \(\text{Ca}^{2+}\) greatly reduced the KCl-induced contraction, as opposed to only moderately attenuating the 5-HT response. This observation is in agreement with the mechanisms of smooth muscle activation proposed for these agents, suggesting that the 5-HT effects are the result of the stimulation of \(\text{Ca}^{2+}\) release from intracellular stores in addition to \(\text{Ca}^{2+}\) mobilization across the plasma membrane.\textsuperscript{21}

The relations between light chain phosphorylation and steady-state stress were different for the two agents. With 5-HT, phosphorylation increased to a high level (0.58 mol phosphate/mol light chain) and then fell to an intermediate value (0.39 mol phosphate/mol light chain), which was maintained for 2 hours of contraction. On the other hand, KCl stimulation resulted in a monotonic increase in phosphorylation to \(\sim 0.35\) mol phosphate/mol light chain, which was also maintained throughout the 2-hour period of stimulation. Whereas each agent resulted in similar, elevated levels of light chain phosphorylation, steady-state stresses were significantly greater in the KCl-depolarized muscles. This is in marked contrast to the bovine trachealis muscle, in which the agonist carbachol elicits significantly greater stress than does KCl.\textsuperscript{22}
V_{0.5} of the bovine coronary arterial smooth muscle were in the range of values reported for hog carotid artery.\textsuperscript{4} These values are significantly less than those measured in the bovine trachealis at equivalent levels of myosin light chain phosphorylation (e.g., 0.05 muscle length/sec versus 0.22 muscle length/sec at 0.6 mol phosphate/mol light chain).\textsuperscript{7} The reasons for large differences in shortening rates independent of light chain phosphorylation among different smooth muscle types, particularly within a single species, are not entirely understood. Myosins purified from different smooth muscle sources have been studied in motility assays in vitro, where myosin-coated bead movement on an actin substratum is believed to correlate with unloaded shortening velocity of muscle fibers.\textsuperscript{23} When evaluated under these conditions, the velocities of beads coated with bovine aortic myosin were consistently lower (0.10 versus 0.13 mm/sec) than those coated with bovine tracheal myosin.\textsuperscript{23} This may indicate that a portion of the difference between shortening velocities is due directly to differences in the properties of myosin itself. However, the in vitro results do not account for fourfold differences seen in vivo, suggesting that additional factors associated with particular muscle types may affect shortening velocity.

Every type of smooth muscle studied to date exhibits variation in crossbridge cycling rates, as reflected by families of force-velocity relations observed under different conditions of activation. V_{0} has been shown for a number of cases to be linearly proportional to crossbridge phosphorylation as reviewed by Hai and Murphy,\textsuperscript{4} and this relation has been formally modeled by the application of A.F. Huxley’s formulation of crossbridge cycling to the latchbridge hypothesis.\textsuperscript{6} A linear relation between myosin light chain phosphorylation and V_{0} was similarly described for neurally stimulated bovine tracheal smooth muscle.\textsuperscript{7} In the bovine coronary artery, V_{0} varied in parallel with phosphorylation; for example, V_{0} exhibited a transient when phosphorylation showed a transient with 5-HT, and V_{0} did not decline significantly when values of phosphorylation were invariant with KCl. For each agonist, the data suggested a linear relation between light chain phosphorylation and V_{0} that intercepted zero velocity at basal levels of phosphorylation (Figure 3). These relations (e.g., data at lower levels of light chain phosphorylation) are not more thoroughly described because of the difficulty of obtaining accurate force-velocity relations at low forces during force development. Nevertheless, it is clear from Figure 3 that the phosphorylation-velocity relations are not the same for the two agonists. It has been shown for isolated smooth muscle myosin that phosphorylation of the regulatory light chain at sites other than that immediately phosphorylated by myosin light chain kinase (serine 19) can alter the actin-activated MgATPase activity of myosin, which would be expected to affect shortening velocity (reviewed in Kamm and Stull).\textsuperscript{24} However, in vitro motility assays\textsuperscript{23} and skinned smooth muscle experiments\textsuperscript{25} have shown that such phosphorylations do not alter velocities of movement or shortening, respectively. Additionally, in the intact coronary arterial muscle, no evidence was found for a difference in sites of phosphorylation under conditions in which velocity varied in the presence of a constant level of phosphorylation. These results and those of others raise the interesting possibility that factors in addition to myosin light chain phosphorylation may modify crossbridge cycling rates within a given muscle.\textsuperscript{26–28} Siegel and colleagues\textsuperscript{28} showed in the Taenia coli that V_{0} was increased with high external Ca\textsuperscript{2+}, whereas light chain phosphorylation was unaltered. They suggested that crossbridge cycling rates in mammalian smooth muscle could be modulated by a calcium-dependent process in addition to myosin phosphorylation. Haeberle and colleagues\textsuperscript{29} showed in permeabilized uterine muscle that shortening velocity undergoes a temporal transient when light chain phosphorylation is invariant at 1.0 mol thiophosphate/mol light chain. The thin-filament protein caldesmon was implicated in this response in that addition of anti-caldesmon antibodies abolished the observed velocity transient. A second thin-filament protein, calponin,\textsuperscript{30} has been shown to inhibit actomyosin ATPase activity,\textsuperscript{31,32} and this inhibition is reversed by calcium/calmodulin or calcium-dependent phosphorylation. Whether Ca\textsuperscript{2+}, caldesmon, and/or calponin are involved in altering V_{0} under constant conditions of crossbridge phosphorylation in the coronary artery remains to be determined.

A better understanding of the processes involved in coronary arterial smooth muscle activation, particularly as these relate to events involved in regulation of the intracellular contractile apparatus, may provide important information in the study of normal and altered physiological function.

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References


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