

Substrate and Product Dependence of Force and Shortening in Fast and Slow Smooth Muscle

MIA LÖFGREN, ULF MALMQVIST, and ANDERS ARNER

From the Department of Physiological Sciences, Lund University, Tornavägen 10, BMC F11, S-22184 Lund, Sweden

ABSTRACT To explore the molecular mechanisms responsible for the variation in smooth muscle contractile kinetics, the influence of MgATP, MgADP, and inorganic phosphate (P_i) on force and shortening velocity in thio-phosphorylated “fast” (taenia coli: maximal shortening velocity $V_{max} = 0.11$ ML/s) and “slow” (aorta: $V_{max} = 0.015$ ML/s) smooth muscle from the guinea pig were compared. P_i inhibited active force with minor effects on the V_{max} . In the taenia coli, 20 mM P_i inhibited force by 25%. In the aorta, the effect was markedly less (<10%), suggesting differences between fast and slow smooth muscles in the binding of P_i or in the relative population of P_i binding states during cycling. Lowering of MgATP reduced force and V_{max} . The aorta was less sensitive to reduction in MgATP (K_m for V_{max} : 80 μ M) than the taenia coli (K_m for V_{max} : 350 μ M). Thus, velocity is controlled by steps preceding the ATP binding and cross-bridge dissociation, and a weaker binding of ATP is not responsible for the lower V_{max} in the slow muscle. MgADP inhibited force and V_{max} . Saturating concentrations of ADP did not completely inhibit maximal shortening velocity. The effect of ADP on V_{max} was observed at lower concentrations in the aorta compared with the taenia coli, suggesting that the ADP binding to phosphorylated and cycling cross-bridges is stronger in slow compared with fast smooth muscle.

KEY WORDS: myosin isoforms • phosphate • ATP • ADP • force-velocity relation

INTRODUCTION

The contractile apparatus in smooth muscle is characterized by lack of sarcomere units, slow contractile kinetics, and myosin-based regulation. Although smooth muscles share these properties, a large heterogeneity in contractile properties exists within the smooth muscle family. The smooth muscles have been divided into “visceral” and “multi-unit” types (Bozler, 1941) or into “phasic” and “tonic” types (Somlyo and Somlyo, 1968) based on the characteristics of the contractile patterns of the intact smooth muscle. Most likely the membrane properties and excitation pathways are coordinated with the properties of the contractile machinery, and as pointed out by Drs. Somlyo and co-workers (Horiuti et al., 1989), the phasic and tonic muscle types have different turnover characteristics of the actin–myosin interaction. The difference in shortening velocity and rate of tension development between fast and slow smooth muscles is equal to, or even greater than, that between fast and slow skeletal muscle types (Malmqvist and Arner, 1991). It is an open question whether smooth muscle can be divided into discrete groups or if the smooth muscle cells and tissues exhibit a continuous distribution in cross-bridge turnover kinetics. A division of smooth muscle into well-defined muscle types (e.g.,

based on kinetics of the actin–myosin interaction) would be important, but is not available at the present.

The molecular mechanisms responsible for the difference in cross-bridge kinetics between smooth muscles are not resolved. Alterations in the loop 1 at the 25/50-kD junction of the myosin II molecule alter the kinetics of myosin (Sweeney et al., 1998). Evidence from in vitro motility experiments shows that a seven amino acid insert in this region modulates the filament velocity and duty cycle of the actin–myosin interaction of smooth muscle (Kelley et al., 1992; Rovner et al., 1997; Lauzon et al., 1998). This effect has been difficult to clearly demonstrate in the organized contractile system of smooth muscle. Comparative studies on smooth muscle preparations have reported a correlation between contractile kinetics and expression of the myosin heavy chain insert as well as with the isoform distribution of the essential light chains of myosin (Malmqvist and Arner, 1991; Fuglsang et al., 1993; Sjuve et al., 1996; Matthew et al., 1998). Forced expression experiments in chicken embryonic smooth muscle cells suggest that the essential light chains can modulate contraction kinetics (Huang et al., 1999). Although the myosin heavy chain insert, and/or light chain isoforms, influence the smooth muscle myosin kinetics, it is unknown which reactions in the cross-bridge cycle determine the kinetics of the organized contractile system in fast and slow smooth muscles.

The actin–myosin interaction in smooth muscle is considered to occur according to the general kinetic

Address correspondence to Anders Arner, MD, Ph.D., Department of Physiological Sciences, Lund University, Tornavägen 10, BMC F11, S-22184 Lund, Sweden. Fax: 46-46-222-7765; E-mail: Anders.Arner@mphyl.lu.se

scheme proposed for skeletal muscle, although several of the rate constants are slower in smooth muscle (Marston and Taylor, 1980; Cremo and Geeves, 1998). One important property of smooth muscle myosin is the high affinity of ADP for the actin–myosin complex *in vitro* (Cremo and Geeves, 1998). Recently, ADP binding to the subfragment 1 of smooth muscle myosin has been shown to induce structural changes in the myosin molecule (Whittaker et al., 1995; Gollub et al., 1996), and kinetic and structural data from smooth muscle myosin suggest the presence of two different actin-myosin-ADP states on the pathway to the ADP release (Rosenfeld et al., 2000). The low, micromolar, dissociation constant for ADP in smooth muscle was first demonstrated in smooth muscle fibers in rigor using competition with pyrophosphate or ATP (Arheden and Arner, 1992; Nishiye et al., 1993). In a study of smooth muscle preparations in rigor, ADP binding did not alter force (Dantzig et al., 1999), suggesting that the structural changes in the isolated myosin molecule upon ADP binding are not readily detected in the organized contractile system. However, a recent report has suggested that mechanical changes can be induced in rigor (Khromov et al., 2001).

The finding that ADP inhibits the rate of relaxation from active contractions (Khromov et al., 1996) suggests that ADP binds strongly to cross-bridge states present during active cross-bridge cycling and that a high ADP affinity may have a functional role in modulating mechanics of active contractions. The ADP release/binding reactions are generally considered to occur at the end of, or after, the cross-bridge working stroke (Cooke and Pate, 1985), and the release of ADP is considered to be rate-limiting for shortening velocity (Siemankowski et al., 1985). Thus, it is possible that the tight ADP binding of the actin–myosin complex has a key role in determining contractile kinetics of smooth muscle.

We have previously shown that ADP inhibits shortening velocity in Ca^{2+} -activated skinned smooth muscle (Arner et al., 1987), but quantitative data regarding ADP effects on the force-velocity relation in a fully activated smooth muscle have not been presented. Differences in contractile kinetics between fast (phasic) and slow (tonic) smooth muscles have been suggested to be due to differences in ADP binding (Fuglsang et al., 1993; Khromov et al., 1996). However, these studies have compared muscles in rigor and in the dephosphorylated state during relaxation. To answer the question whether reactions involved in binding or release of ADP, or possibly of ATP or phosphate, are responsible for the difference in shortening velocity between fast and slow smooth muscles, we have performed a detailed study of the interaction between actin and myosin in the organized contractile system by determining force-velocity relationships in fully thiophosphorylated

preparations of a fast (taenia coli) and a slow (aorta) smooth muscle from guinea pigs at different concentrations of ADP, ATP, and phosphate. Preliminary results of this work have been presented previously (Malmqvist et al., 1999).

MATERIALS AND METHODS

Muscle Preparations

The taenia coli and thoracic aorta were obtained from female guinea pigs. The animals weighed ~ 400 g and were killed by cervical dislocation. The muscle tissues were cut out under a microscope and chemically skinned using Triton X-100 as described by Arner and Hellstrand (1985). The preparations were then stored at -18°C until they were used. Immediately before each experiment, fiber preparations were cut out. The preparations from the taenia coli had a diameter of ~ 0.15 mm and a length of 2–4 mm. A 1.2–1.8-mm-long segment of the aorta was cut open and mounted with the circular muscle layer in the long axis of the preparation, which gave preparations of 2–3-mm length with the full thickness of the media layer (~ 0.05 mm).

Solutions

The solutions used for the skinned muscle preparations contained 30 mM *N*-Tris-(hydroxymethyl)methyl-2-aminoethane-sulfonic acid, 4 mM EGTA, and 2 mM free Mg^{2+} . All solutions were adjusted to pH 6.9 with KOH and to an ionic strength of 150 mM using KCl. In the experiments, the concentrations of MgATP, MgADP, and inorganic phosphate (P_i)¹ were varied. When ADP-depletion/ATP-generation was used, 12 mM phosphocreatine (PCr) and 0.5 mg/ml creatine kinase (CK) were added to solutions or, in the ATPase-determining experiments, 10 mM phosphoenolpyruvate and 20 U/ml pyruvate kinase. Experiments using ADP were performed in the presence of 0.2 mM of the myokinase inhibitor AP_5A (Feldhaus et al., 1975). The standard relaxation and activation solutions contained 3.2 mM MgATP. The composition of all solutions was calculated using a computer program and stability constants essentially as described by Fabiato and Fabiato (1979) and Fabiato (1981). All experiments were performed at room temperature (22°C). All chemicals were purchased from Sigma-Aldrich and Boehringer. Calmodulin was a gift from Dr. E. Thulin (Department of Physical Chemistry II, Lund University).

Quick-release Experiments

The muscle preparations were wrapped with small clips of aluminum foil at each end and mounted between a stainless steel pin attached to a force transducer (model AE 801; SensoNor a.s.) and an isotonic lever (Arner and Hellstrand, 1985). The lever arm could be released with electromagnetic relays. The afterload on the muscle was determined by a spring load on the lever. After release, a stable afterload was established within ~ 5 ms. After each quick release, force and muscle length were recorded for 1 s using a sampling rate of 1 kHz on an RTI-800 Analogue Devices A/D board in a personal computer. The shortening velocity was determined at different points in time after the release by analysis of the length responses as described by Arner (1982). The length of the muscle was measured at the end of the experiment using a microscope with an ocular scale and the velocity values were expressed in muscle lengths (ML) per second. The following Hill (Hill, 1938) equation was fitted to the force and velocity (V) data:

¹Abbreviations used in this paper: A-M, actin-myosin; A-M-ADP, actin-myosin-ADP; CK, creatine kinase; ML, muscle lengths; PCr, phosphocreatine; P_i , inorganic phosphate.

$$V = b(1 - P/P_o)/(P/P_o + a/P_o). \quad (1)$$

In Eq. 1, a and b are constants, P the afterload, and P_o the isometric force at each contraction. The maximal shortening velocity (V_{max}) was then given by extrapolation of the fitted curve to $P/P_o = 0$.

The preparations were initially mounted in a relaxing solution (1 nM free $[Ca^{2+}]$, pCa 9) and stretched to a passive force of ~ 0.1 mN. Thereafter, the muscles were maximally activated using a repeated thiophosphorylation procedure (Arheden et al., 1988). Thiophosphorylation of the regulatory light chains was done by treating the muscle for 10–15 min using a calcium-containing (pCa 4.5) rigor solution with 0.5 μ M calmodulin and 1.8 mM ATP- γ -S. After a 5-min period in calcium-free rigor solution, a contraction was initiated by transfer to an ATP containing solution. When force had reached a plateau, the muscle fiber was transferred to fresh solution and a series of 15–30 releases to different afterloads was performed. The isometric force was measured immediately before the beginning of each release series. Before the next contraction and force-velocity determination, the fiber was again treated with thiophosphorylation. A maximum of five (taenia coli) and six (aorta) contractions and force-velocity determinations were made on each preparation. In general, force and velocity data were normalized to values obtained in each fiber preparation during a standard reference contraction at saturating (3.2 mM) $[MgATP]$ in the presence of PCr and CK. The different solutions were applied at random order. Six sets of experiments were performed: (1) varied $[MgATP]$ in the presence of the PCr/CK system; (2) varied $[MgATP]$ in the absence of the PCr/CK system; (3) varied $[MgATP]$ in the presence of MgADP in the absence of the PCr/CK system (taenia coli only); (4) varied $[MgADP]$ at constant $[MgATP]$ (taenia coli: 1 and 6 mM; aorta: 10 mM); (5) 0 and 20 mM P_i at 3.2 mM $MgATP$ in the presence of the PCr/CK system (aorta only); and (6) in rigor solutions (0 mM $MgATP$ with 50 U/ml hexokinase and 10 mM glucose). In some of these experiments, apyrase (20 mg/ml) was included in rigor solutions.

For analysis of the $[MgATP]$ dependence of the maximal shortening velocity (V_{max}), a hyperbolic equation was used to determine the apparent binding constant (K_m). Max denotes the V_{max} at saturating $[MgATP]$.

$$V_{max} = \text{Max} \cdot [MgATP]/(K_m + [MgATP]). \quad (2)$$

For analysis of $[MgADP]$ dependent inhibition of velocity, the following equation was used to determine the apparent inhibition constant (K_i):

$$V_{max} = (\text{Max} \cdot [MgATP]) / (K_m(1 + [MgADP]/K_i) + [MgATP]). \quad (3)$$

Isometric Force Experiments

In one series of experiments, the influence of varied P_i concentrations on active force was determined in the taenia coli and aorta. The preparations were mounted in 0.5-ml plastic baths for isometric force registration using AE 801 force transducers. Activation with thiophosphorylation was performed as described above. Four preparations were studied in parallel and force was measured at P_i concentrations in the range 0–40 mM in solutions with 3.2 mM $[MgATP]$ and PCr/CK system. P_i was introduced in the contraction solution and force was determined at the plateau of each active contraction.

Determination of Tissue ATPase Activity

In a series of experiments performed to examine ATPase activity, skinned muscle fibers from the taenia coli and the aorta were mounted in 0.5-ml cups and attached to Grass FT03 force transducers. The experiments were performed essentially as described by Arner and Hellstrand (1985). The fibers were incubated in contraction and relaxation solutions containing phosphoenolpyruvate and pyruvate kinase for 15 min. Incubation solutions and blanks were frozen. The release of pyruvate, which is proportional to the production of ADP, was determined using a fluorimetric assay. The values from the incubation solutions and the blanks were compared with a standard curve with known concentrations of ADP. ATPase activity was related to the wet weight of the preparations.

Determination of Myosin Isoforms

To examine the content of myosin essential light chain 17a and 17b in the skinned taenia coli and the aorta fibers (compare with Malmqvist and Arner, 1991), the proteins were separated according to isoelectric point (first dimension), using ampholytes in the pH range 4.5–6. For the second dimension, the proteins were separated according to molecular mass using 15% polyacrylamide gels. The gels were stained with Coomassie blue and evaluated with densitometric scanning.

Statistics

All values are given as mean \pm SEM with the number of observations within parenthesis. Curve fitting to the hyperbolic Hill (1938) force-velocity relation was performed using a nonlinear procedure to minimize the perpendicular distance between data points and the fitted curve (Fletcher and Powell, 1963) as described previously (Arner, 1982, 1983). All other curve fitting was performed using the nonlinear fitting routines implemented in Sigmaplot for Windows (Jandel Corp.).

RESULTS

Fig. 1 shows original recordings of force from quick-release experiments on a taenia coli and an aorta preparation. Contractions were elicited in the thiophosphorylated preparations at different MgADP concentrations at 1 and 10 mM $MgATP$ for the taenia and the aorta, respectively. The different MgADP concentrations were applied at random order. Experiments with varied $MgATP$ were performed in a similar way. We have previously shown (Arheden et al., 1988) that the protocol with repeated thiophosphorylation gives reproducible contractions with $<5\%$ difference in maximal shortening velocity (V_{max}) and force between the first and seventh contraction in the taenia coli muscle. In the present study, we evaluated whether force and velocity were changed with the number of contractions for the aorta and found that V_{max} and force at optimal $[MgATP]$ for the last contraction (No. 3 or 5) in the series were essentially similar to the initial values ($V_{max} = 95 \pm 4$; force = $112 \pm 5\%$ of initial value, $n = 6$).

The force and velocity data from taenia coli and aorta preparations could be described by the Hill (1938) equation (Eq. 1) as shown in Fig. 2. As seen in Fig. 2 B the force-velocity relation of the aorta was less concave compared with that of the taenia coli (Fig. 2 A) especially at

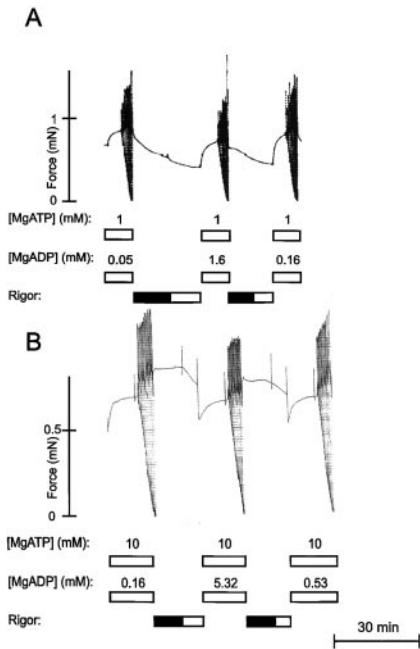


FIGURE 1. Original recordings of force from quick-release experiments on a taenia coli (A) and an aorta (B) preparation. The trace starts after the initial contraction. The regulatory light chains were thiophosphorylated using ATP- γ -S in a rigor solution with pCa 4.5 between contractions (filled bar). After a rinse in Ca^{2+} -free rigor solution, active contractions were elicited by adding MgATP (1 mM for the taenia coli and 10 mM for the aorta) at different MgADP concentrations, indicated below the records. The transients on the force records are due to the releases to different afterloads used to determine the force-velocity relation.

low MgATP concentrations, which corresponds to high a/P_o and low b values in the Hill (1938) equation. The variation in the parameters a/P_o and b was large, and their interpretation in smooth muscle is unclear. Therefore, we did not analyze these parameters of the Hill equation. The fitted Hill equation deviated very little

from the data points at lower relative afterloads, and we used the equation to obtain estimates of the V_{\max} .

The shortening response after a quick release in smooth muscle consists of an initial elastic recoil followed by a shortening with gradually decreasing velocity (Arner, 1982; Arner and Hellstrand, 1985). In accordance with our previous analysis (Arner and Hellstrand, 1985), we used the velocity values determined 100 ms after release. Velocity values presented here refer to this time point, unless stated otherwise.

We have previously reported (Malmqvist and Arner, 1991) that the guinea pig taenia coli has $\sim 20\%$ of the basic essential myosin light chain (LC_{17b}). This was confirmed in the present study ($\sim 25\%$ LC_{17b}). Previous studies have reported $\sim 60\%$ LC_{17b} in rat and rabbit aorta (Malmqvist and Arner, 1991) and in rabbit femoral artery (Fuglsang et al., 1993). In the present study, we found $\sim 70\%$ LC_{17b} in the guinea pig aorta, which is consistent with the previous data from large elastic arteries.

We observed, when we determined the [MgATP] dependence of V_{\max} that the relation did not extrapolate to zero V_{\max} at zero [MgATP]. Therefore, we also performed quick-release experiments in rigor and found a significant shortening with an apparent V_{\max} under these conditions. The V_{\max} in rigor at 100 ms after release, relative to that at optimal [MgATP], was $39 \pm 1.2\%$ ($n = 14$) for the taenia coli and $55 \pm 3.5\%$ ($n = 7$) for the aorta. Quick-release experiments on preparations fixed with glutaraldehyde at the end of the experiments showed no shortening response, excluding that the shortening observed in rigor muscles was due to compliance of transducer, lever arm, or fiber attachment. A significant shortening response in rigor muscles was also observed in preparations where the ends had been fixed with cellulose acetate glue and using the slack test method. This shows that the response in rigor was not due to the aluminum clips or to the iso-

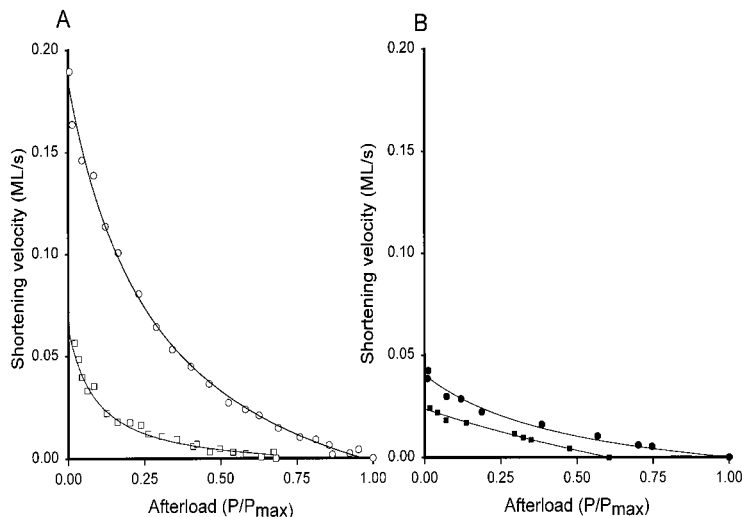


FIGURE 2. Force-velocity relationships of a taenia coli (A) and an aorta (B) preparation. Shortening velocity in muscle lengths (ML) per second is plotted against the relative afterload (P/P_{\max}), where P_{\max} is the isometric force at 3.2 mM MgATP. The diagrams show relationships at 3.2 mM (circles) and 0.01 mM MgATP (squares) in the presence of the ATP backup system. The Hill force-velocity equation (Eq. 1; see MATERIALS AND METHODS) was fitted to the data and extrapolated to $P/P_{\max} = 0$ to obtain the extrapolated maximal shortening velocity. The maximal unloaded shortening velocity for the taenia coli was 0.174 ± 0.006 ML/s ($n = 13$) and for the aorta was 0.036 ± 0.002 ML/s ($n = 8$). Note that these values are not corrected for the viscous component (see RESULTS).

tonic quick-release method. To ensure that the shortening in rigor was not due to ATP contamination in the solutions the rigor experiments were performed in solutions supplemented with glucose/hexokinase and apyrase as described in MATERIALS AND METHODS. To further ensure that shortening in rigor was not due to active cross-bridge cycling, we performed experiments in the presence of 1 mM vanadate, an inhibitor of active cross-bridge cycling in smooth muscle (Jaworowski et al., 1999). The force and V_{\max} in rigor were unchanged when vanadate was added in the aorta ($n = 2$) and taenia coli ($n = 2$). Addition of 5.32 mM MgADP to rigor did not alter the maximal shortening velocity in rigor ($n = 2$) in the aorta.

We have interpreted the shortening in rigor conditions as a result of viscous phenomena in the preparation. Since passive force in the relaxed state (pCa 9 solution) was low (taenia coli: 0.7 ± 0.5 ; aorta: $4.3 \pm 1\%$ of maximal active tension, $n = 10$), elastic or viscous elements in parallel with the contractile apparatus would not contribute to force or shortening. Therefore, we assumed a visco-elastic element in series with the contractile component. The shortening responses in rigor after a quick release consisted of an initial elastic recoil, and a subsequent "viscous" phase of slower shortening. The calculated maximal shortening velocity in rigor decreased with time in an approximately similar manner as in the active contraction (V_{\max} at 500 ms after release relative to that at 100 ms, taenia coli rigor: 20.1 ± 1.5 ; aorta rigor: 20.6 ± 3.2 ; taenia coli active: 31.9 ± 0.7 ; aorta active: $23.6 \pm 1.7\%$, $n = 6$). Thus, the time constant of the visco-elastic element is approximately the same as that of the active response. Therefore, the visco-elastic component cannot be simply eliminated by measuring velocity at a different time after release. The amplitude of the viscous shortening phase in rigor, which reflects the properties of the spring in the visco-elastic

component, was not linearly dependent on the amplitude of the force step. The resulting strain-force relationship was nonlinear, with an increasing stiffness at increasing strain. The behavior could be adequately described by an exponential spring similar to that proposed for series and parallel elastic components (Arner and Hellstrand, 1985). A simple visco-elastic element, composed of a viscous element and a linear spring, would have a linear dependence of velocity on force. In a visco-elastic element containing an exponential spring, the dependence of velocity on force becomes complex. Using an analytical solution to the differential equation describing a model with an element in series with the contractile component composed of a linear viscous element (force is proportional to velocity) and a nonlinear (exponential) spring in parallel (Voigt configuration), we simulated the dependence of maximal velocity on the isometric tension before release. The model predicts that the relation between maximal shortening velocity and isometric tension is nonlinear; velocity determined at higher isometric tension levels has a very weak dependence on force. The rigor isometric force in our experiments was in the range 28–55% (taenia coli) and 60–78% (aorta) of maximal active isometric force (at 3.2 mM MgATP). Linear regression of the data gave a very weak dependence of V_{\max} on force (taenia coli: $r^2 = 0.26$ and aorta: $r^2 = 0.02$) with curves that intercepted with the velocity-axis clearly above zero velocity. These data are consistent with the predictions of the model discussed above. Based on these considerations, an estimate of the V_{\max} of the contractile component can be obtained by subtracting the rigor V_{\max} , without correction for the isometric force level. Using this correction the [MgATP] dependence of V_{\max} extrapolated to zero velocity at zero [MgATP] and was adequately described by a hyperbolic equation (Eq. 2), as discussed below (Fig. 3). After correction, the V_{\max} of

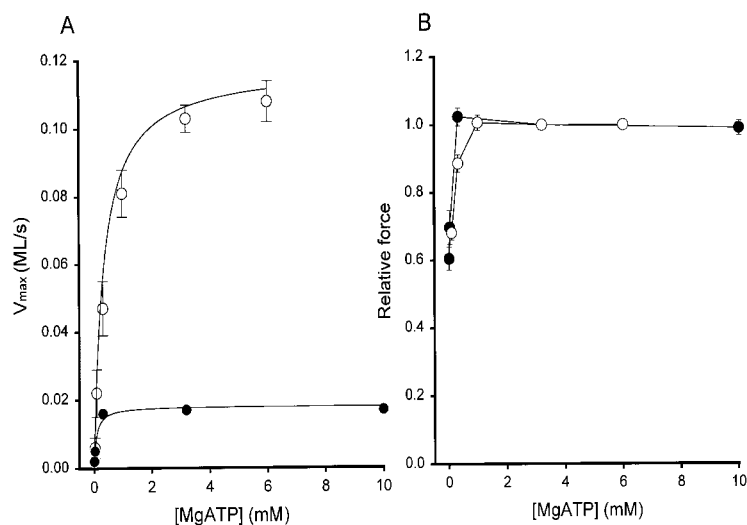


FIGURE 3. The dependence of maximal shortening velocity in muscle lengths per second (A) and of force, relative to force at optimal [MgATP], (B) on the MgATP concentration in taenia coli (open circles) and aorta (closed circles). A hyperbolic equation (Eq. 2; see MATERIALS AND METHODS) was fitted to the whole material of velocity data to obtain the apparent K_m of the maximal shortening velocity (V_{\max}) for MgATP. K_m was 351 ± 76 and $84 \pm 31 \mu\text{M}$ for the taenia coli and the aorta, respectively ($n = 5-7$).

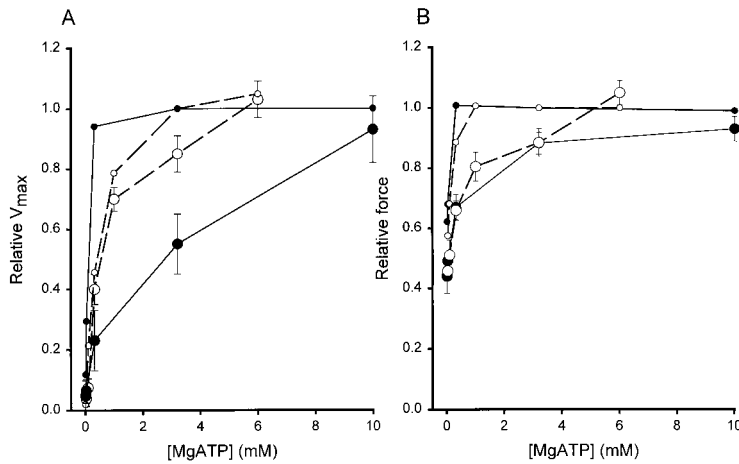


FIGURE 4. The [MgATP] dependence of V_{\max} (A) and force (B), in the absence of the PCr/CK-backup system for aorta (closed symbols, full line) and taenia coli (open symbols, dashed line). The values are expressed relative to those at 3.2 mM [MgATP] in the presence of PCr and CK. Dashed and full lines with small symbols show, for comparison, the [MgATP] dependence of V_{\max} with PCr/CK-backup, for the taenia coli and the aorta, respectively (data from Fig. 3; $n = 5-7$).

the taenia coli was 0.11 ML/s and for the aorta 0.015 ML/s. This makes the difference in V_{\max} between the two muscle types slightly greater than evident in Fig. 2. In the following data presentation, we have subtracted the rigor velocity from the V_{\max} values.

The results in Fig. 3 A show the ATP dependence of V_{\max} for aorta and taenia coli preparations. The relation for the taenia coli was shifted towards higher [MgATP] compared with the aorta and the apparent K_m (Eq. 2) was about fourfold higher. A fit to the whole data set gave K_m values of $351 \pm 76 \mu\text{M}$ for the taenia coli and $84 \pm 31 \mu\text{M}$ for the aorta. Fig. 3 B shows the corresponding force values. Force was lower at reduced [MgATP], but appeared to be less influenced by a change in [MgATP] than V_{\max} . Force in rigor was 0.69 ± 0.03 (6) for the aorta and 0.42 ± 0.02 (14) for the taenia coli, relative to the corresponding values at optimal (3.2 mM) [MgATP]. In separate experiments, we determined active force normalized to cross-sectional area (determined by dividing preparation wet weight with length and density) and found that the force of the aorta preparations at maximal activation and optimal [MgATP] was lower than that of the taenia coli (4.5 ± 0.8 (6) vs. 59 ± 19 (4) mN/mm²). It should be noted that these values were not corrected for tissue content of smooth muscle myosin. The absolute forces that the preparations developed in these experiments were 6.4 ± 0.8 (4) mN (taenia coli) and 1.7 ± 0.3 (6) mN (aorta).

As shown in Fig. 4 A, the V_{\max} of the skinned muscles was dependent on the presence of the phosphocreatine/creatine kinase system (PCr/CK). Removal of the backup system resulted in a significant reduction of the maximal shortening velocity at lower ATP concentrations. This effect was more pronounced in the aorta compared with the taenia, showing that lowered ATP/ADP ratios in the muscle fiber influence velocity more in the slow smooth muscle. The apparent K_m for MgATP increased ~ 1.8 -fold in the taenia coli and ~ 34 -fold in the aorta.

In experimentation without backup system, diffusion of ATP and ADP in the preparations becomes important. Most likely the change in ATP dependence of V_{\max} , observed when the backup system is removed (Fig. 4) is due to a change in the ADP/ATP ratio in the interior of the fiber preparation. To exclude that the more pronounced dependence on the backup system in the aorta preparations was due to greater changes in ADP/ATP in the tissue due to higher tissue ATPase, we examined the tissue ATPase activity in the presence of phosphoenol pyruvate. In the maximally thiophosphorylated preparations, the ATPase was 0.51 ± 0.12 (4) $\mu\text{mol min}^{-1} \text{g}^{-1}$ for the taenia coli and 0.31 ± 0.02 (6) $\mu\text{mol min}^{-1} \text{g}^{-1}$ for the aorta. These values reflect the total ATPase in the active muscle and most likely include contribution from both actin-myosin interaction and non-contractile, possibly ecto-, ATPases in the tissue. Assuming a cylindrical geometry and a diffusion constant of $2 \times 10^{-7} \text{ cm}^2/\text{s}$ (Mannherz, 1968) the approximate [MgADP] and [MgATP] in the center of the taenia coli preparations could be calculated from preparation dimensions and ATPase (Cooke and Pate, 1985). At 3.2 mM MgATP in the bathing medium, [MgATP] and [MgADP] in the center on the taenia coli were calculated to 2.6 and 0.6 mM, respectively. Since the aorta preparation was thinner and had a lower ATPase activity, the concentrations in the center of the preparation would be similar to those in the bathing medium (3.2 and 0.04 mM for [MgATP] and [MgADP], respectively) when calculated as above. These calculations show that the differences in dependence of the backup system between the aorta and the taenia coli preparations are not due to differences in tissue ATPase or ADP/ATP diffusion, but rather reflect a difference in ATP binding or sensitivity to ADP as discussed below.

To investigate the effects of ADP on the ATP dependence of V_{\max} , force-velocity relations were determined at different ATP concentrations in the presence of 2.66 and 5.32 mM MgADP in the taenia coli preparation

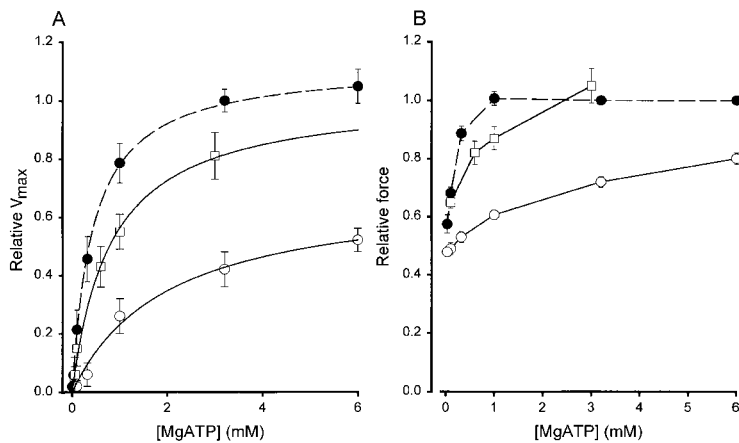


FIGURE 5. Effects of MgADP on the MgATP dependence of the maximal shortening velocity (V_{\max}) and force in the taenia coli. V_{\max} and force, relative to the values at 3.2 mM MgATP and PCr/CK, are plotted against the MgATP concentration at 0 (with PCr/CK, closed circles, dashed line, same data as in Fig. 3), 2.66 mM MgADP (without PCr/CK, open squares) and 5.32 mM MgADP (without PCr/CK, open circle). The curves in A show fits to a hyperbolic equation (Eq. 2) to the mean values, giving apparent K_m values for MgATP of 0.460 (0 mM MgADP), 0.811 (2.66 mM MgADP), and 1.998 mM (5.32 mM MgADP; $n = 6-8$).

(Fig. 5). The ATP dependence of V_{\max} (Fig. 5 A) was shifted towards higher [MgATP] in the presence of ADP. Addition of 2.66 mM [MgADP] did influence the extrapolated V_{\max} at saturating [MgATP] to a minor extent and the apparent K_m for MgATP increased to 0.811 mM (fit to Eq. 2). The inhibition of V_{\max} at the high MgADP concentrations (5.32 mM) could not be reversed by increased [MgATP], suggesting noncompetitive effects of ADP at higher concentrations. Increasing [MgADP] resulted in a decrease in active force (Fig. 5 B).

Experiments using addition of ADP have to be performed in the absence of a backup system. Since V_{\max} of the aorta was markedly influenced by removal of the backup system (Fig. 4 A), it is not possible to examine the effects of varied [MgATP] at constant [MgADP] in this preparation as was performed for the taenia coli (Fig. 5). Instead, we chose concentrations of MgATP (6 mM for the taenia coli and 10 mM for the aorta) where removal of the backup system did not influence the maximal velocity or force (Fig. 4). At these MgATP concentrations, we varied the [MgADP]. Fig. 6 shows the effects of ADP on the maximal shortening velocity and force of aorta and taenia coli preparations. Addition of ADP inhibited V_{\max} in both tissues, but the effects occurred at much lower concentrations in the aorta. Note that [MgATP] was higher in the experiments on the aorta, showing that the inhibition of velocity occurred at lower ADP/ATP ratios. The velocity did not approach zero even at high [MgADP], but approached a value of $\sim 50\%$ of maximal. This behavior was observed both in the aorta and the taenia coli (Fig. 6). Even at lower [MgATP] (1 mM, data not shown), addition of ADP did not reduce velocity to zero in the taenia coli; V_{\max} was inhibited at saturating [MgADP] to $\sim 50\%$ of the value at zero MgADP. The data for the whole range of ADP concentrations in Fig. 6 could not be directly described using simple Michaelis-Menten's kinetics since velocity was not inhibited to zero at saturating ADP. If we only use the initial part of the data at nonsaturating ADP concentrations (where Lineweaver-Burke

plots were linear) fitting to Eq. 3 gave apparent K_i values for ADP of $\sim 10 \mu\text{M}$ in the aorta and $\sim 360 \mu\text{M}$ in the taenia coli. Although the K_i values at present cannot be directly interpreted, the analysis show that a pronounced difference in the ADP binding exists between the two muscles. In the aorta, force was slightly inhibited in the highest MgADP concentration interval, but was essentially unchanged in the range of MgADP concentrations where the inhibition of velocity occurred. Thus, velocity could be decreased by ADP by $\sim 30\%$, at essentially unchanged force in the aorta.

Fig. 7 shows the effects of P_i on active force and shortening velocity. Force was inhibited by P_i in a dose-dependent manner in both aorta and taenia coli. The effects of phosphate were more pronounced in the taenia coli preparations. The relation between force and $\log_{10}([P_i])$ was almost linear. The effects of phosphate on maximal shortening velocity were investigated in

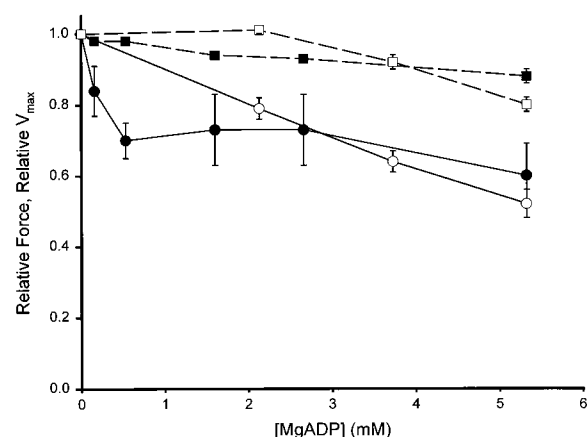


FIGURE 6. Maximal shortening velocity and force at different MgADP concentrations. Force (squares, dashed lines) and maximal shortening velocity (circles, full lines) for the taenia coli (open symbols, 6 mM MgATP) and aorta (closed symbols, 10 mM MgATP) are plotted against the MgADP concentration. Force and V_{\max} are expressed relative to the values at 0 MgADP (3.2 mM MgATP, without PCr/CK backup; $n = 5-10$).

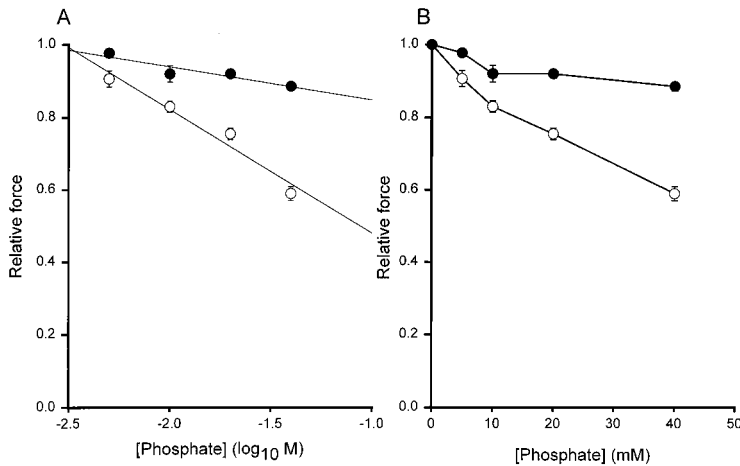


FIGURE 7. Effects of P_i on active force in the aorta and the taenia coli. $[P_i]$ dependence of isometric force of taenia coli (open circles) and aorta (closed circles). Force data plotted against (A) $\log_{10}([P_i])$ and (B) $[P_i]$. Force values are normalized to the force in the absence of P_i .

the aorta preparations. In the presence of 20 mM P_i , where force was inhibited by $\sim 15\%$, velocity was slightly, but not significantly, increased. Maximal shortening velocities of aorta preparations at 3.2 mM MgATP and PCr/CK were with 20 mM P_i 0.017 ± 0.002 ML/s and without P_i 0.014 ± 0.001 ML/s.

DISCUSSION

The aim of the present study was to examine in detail the substrate and product dependence of the force-velocity relation in two smooth muscles that represent the near extremes in the distribution of smooth muscle contractile properties. The guinea pig aorta belongs to the slow group of muscles with high content of essential light chain b (LC_{17b}) and low or lacking heavy chain insert (Malmqvist and Arner, 1991; White et al., 1993), whereas the taenia coli belongs to the fast smooth muscles (Malmqvist and Arner, 1991). The difference in maximal shortening velocity (V_{max}) is about sevenfold (Fig. 3), which is greater than the difference in shortening velocity between fast- and slow-twitch skeletal muscles (e.g., rabbit psoas versus soleus; Pate et al. 1992).

Our quick-release experiments on muscles in rigor revealed an important technical aspect of experiments on smooth muscle preparations, i.e., a significant contribution of viscous elements on the shortening responses. A more detailed analysis revealed that the viscous responses in rigor could be described by a series-coupled element with a nonlinear spring and a viscous element. This model showed a weak dependence of viscous maximal velocity on isometric tension in rigor and, therefore, we subtracted a constant velocity from the active contractions. After this correction, the dependence of V_{max} on $[MgATP]$ could be adequately described by a hyperbolic equation. At present, we do not have any data regarding the nature of the viscous component, it might reside outside of the contractile machinery, in the cytoskeleton, in the cell-cell interactions, or be a part of the cross-bridge interaction.

The phosphate (P_i) release is considered to be associated with force generation in skeletal and smooth muscles (Hibberd et al., 1985; Österman and Arner, 1995). The phosphate release is not rate-limiting for the maximal shortening velocity in the aorta (present study) and the taenia coli (Österman and Arner, 1995), suggesting that the actin-myosin-ADP (A-M-ADP) states binding P_i do not resist shortening and are thus not responsible for the slower velocity in the aorta. Also, V_{max} is not simply a function of force, since a reduction in isometric force (by P_i), does not reduce V_{max} . Thus, the difference in cross-bridge kinetics between smooth muscle tissues does not only involve alterations in reactions rate-limiting for shortening velocity, but also in the P_i -release reactions associated with force generation.

The apparent K_m for the ATP effects on V_{max} in the slow aorta muscle was about fourfold lower than that of the fast taenia coli smooth muscle, with apparent K_m values of 84 and 351 μM , respectively. This finding is consistent with results from slow and fast rabbit skeletal muscles, where the K_m for MgATP was lower in the slow muscle (semimembranous, 18 μM ; psoas, 150 μM ; Pate et al. 1992). If it is assumed that ADP and ATP binds to actin-myosin (A-M) states generated after the power stroke and before detachment, increased ADP and decreased ATP would increase the population of A-M and A-M-ADP states. These states would resist shortening until detached by ADP release and binding of ATP or by direct dissociation by the filament sliding (Cooke and Pate, 1985). If the rate-limiting step for shortening velocity occurs before the ATP-induced detachment, ATP has to be reduced to a lower concentration in a slow muscle to influence shortening velocity. Since the apparent K_m was lower in the slow aorta compared with the taenia coli (Fig. 3, present study) we can thus exclude that differences in the ATP-induced detachment reaction are responsible for the difference in V_{max} between the fast and slow smooth muscles.

Actually a comparison of the ratios V_{\max}/K_m between muscles gives information on the difference in apparent second-order rate constant for ATP-induced dissociation, assuming similar sarcomere equivalent lengths and cross-bridge attachment ranges. This ratio was $\sim 50\%$ lower in the aorta, which could be consistent with a slightly lower dissociation constant for ATP. The second-order rate constant for the ATP-induced dissociation from rigor has been suggested to be lower in smooth muscle fiber preparations compared with skeletal muscle (Somlyo et al., 1988), and has been reported to be about threefold lower in tonic compared with phasic smooth muscles (Khromov et al., 1996). Our data are not inconsistent with a difference in ATP binding, but show that a difference in the ATP-dependent dissociation cannot explain the difference in shortening velocity between fast and slow smooth muscles at least in the physiological range of ATP concentrations.

Previous studies on skinned smooth muscles in rigor have shown a strong MgADP binding, with a K_d of $\sim 1 \mu\text{M}$ (Arheden and Arner, 1992; Nishiye et al., 1993), compared with a K_d of $\sim 60 \mu\text{M}$ in skeletal muscle (Schoenberg and Eisenberg, 1987). Although a strong ADP binding has been demonstrated in dephosphorylated smooth muscles during relaxation from active contractions (Khromov et al., 1996) quantitative data regarding ADP and ATP binding to phosphorylated myosin during cross-bridge cycling have been lacking. Experiments regarding ADP effects in muscle fiber preparations during active contraction are complicated by the fact that ADP-depleting/ATP-generating backup systems cannot be used. The ATPase activity of the smooth muscle is low, our preparations were made small, and we used comparatively high [MgADP] and [MgATP]. Therefore, we predict that effects of gradients are minimal and have used the bath concentrations of the substrates and products in our analysis. The comparatively high MgADP dependence of the slow aorta muscle was not due to gradients created by diffusion or tissue ATPase, since the ATPase activity was lower and dimensions of the aorta preparations were smaller. When we performed experiments on the aorta preparations, removal of the PCr/CK backup system gave a prompt reduction in V_{\max} . Thus, this finding suggests that the shortening of the slow, more economical, aorta is highly dependent on the backup system. Since our data regarding [MgATP] variations show that the apparent K_m for ATP is lower in the slow muscle, the influence of the backup system cannot be explained by ATP depletion in the muscle tissue, but rather by ADP accumulation. Since the ATPase activity of the aorta is lower than that of the taenia coli, the effect of the backup system most likely reflects a significant difference in the effects of [MgADP] on the shortening velocity between the slow and fast smooth muscles.

Even though the inhibition of V_{\max} by MgADP occurred at low concentrations, the velocity was only inhibited to $\sim 50\%$ of maximal at saturating [MgADP] in the presence of MgATP (Fig. 6). This behavior is clearly different from the inhibition of velocity when ATP was reduced, where V_{\max} approached zero (i.e., the apparent V_{\max} in rigor). We assume that addition of ADP generates a population of A-M-ADP states that oppose shortening. It could be possible that the situation at saturating [MgADP] is a rigorlike state with altered viscous properties giving an apparent velocity that is higher than that in rigor. This seems very unlikely since we found that addition of MgADP to rigor did not increase velocity. A second possible explanation could be that the MgADP binding is weaker at higher MgADP concentrations, a situation where velocity is decreased. This finding is difficult to explain since a lower velocity would shift the distribution of cross-bridge strain towards lower strain in the negative direction, which according to general models of muscle contraction would increase the binding affinity of MgADP (Pate and Cooke 1989). A more complex model would be to consider cooperative phenomena between the myosin heads. It has been recently proposed for skeletal muscle that the binding of the two myosin heads to actin occurs in a coordinated cooperative manner (Conibear and Geeves, 1998). Our finding from the smooth muscle that ADP can only inhibit velocity to $\sim 50\%$ might be consistent with a model where only one of the two myosin heads can initiate and perform the power stroke reactions, and where a subsequent attachment of the second head promotes ADP release of the leading head.

The interpretation of the K_i values is not straight forward since we could not completely inhibit shortening velocity and we cannot at this stage present a complete model to analyze the behavior. However, if we analyze the initial part of the ADP inhibition data (Fig. 6) we find an apparent K_i value of $\sim 10 \mu\text{M}$ in the aorta and $360 \mu\text{M}$ for the taenia coli. This suggests that the binding of ADP to cycling cross-bridges differs with a factor of ~ 40 between the slow and fast smooth muscle types. This result is consistent with the finding of a fourfold difference in binding of ADP to rigor cross-bridges between a fast/phasic ($K_d = 4.9 \mu\text{M}$ rabbit bladder) and a slow/tonic ($K_d = 1.1 \mu\text{M}$ rabbit femoral artery) smooth muscle (Fuglsang et al., 1993). The larger difference in our K_i values could reflect a difference in the distribution of strain between the fast (taenia coli) and slow (aorta) smooth muscles during active shortening.

In the in vitro motility assay, the velocity of actin over smooth muscle myosin is influenced by ATP and ADP concentrations. It has been shown that the K_m for ATP is $\sim 40 \mu\text{M}$ and the K_i for ADP is 0.24 mM using turkey gizzard myosin at 30°C (Warshaw et al., 1991). Addition of phosphate at high [MgATP] did not influence the veloc-

ity. These data are generally consistent with our observations in smooth muscle fibers. In comparison, we find a slightly higher K_m for MgATP (351 μ M) and a higher apparent K_i for ADP (360 μ M) for the guinea pig taenia coli muscle fibers at 22°C. It should be recognized that the interaction between actin and myosin in the in vitro motility assay does not mimic all aspects of the actin-myosin interaction in the muscle fiber. In vitro motility assay studies have shown that an insert in the myosin heavy chain influences velocity (Kelley et al., 1992; Rovner et al., 1997; Sweeney et al., 1998) and duty cycle (Lauzon et al., 1998). The velocity variation is about twofold or less in these studies. This is in contrast with the results from smooth muscle fibers where the variation in velocity is more than fivefold (Malmqvist and Arner, 1991; present study). Another difference between the in vitro motility assay and studies of velocity is that the ADP dependence of velocity in in vitro motility assay was similar with and without insert (Rovner et al., 1997), whereas the ADP dependence differs markedly between fast and slow muscles both under isometric and isotonic conditions (Fuglsang et al., 1993; Khromov et al., 1996; present study). Thus, it is likely that the structure and organization of the contractile filaments in the smooth muscle fiber have a major influence of kinetics of the cross-bridge cycle, possibly by altering strain dependence of reactions in the cross-bridge cycle.

In the intact smooth muscle, intracellular [ADP] has been shown to be in the submillimolar range in the relaxed state and to increase during active contraction and metabolic inhibition (Hellstrand and Paul, 1983; Krisanda and Paul, 1983; Hellstrand and Vogel, 1985; Fisher and Dillon, 1988). In the aorta, we find an apparent K_i for ADP and shortening velocity in the micromolar range. Interestingly, the effect of ADP on velocity in the aorta muscle occurred at almost unchanged force (Fig. 6), a phenomenon similar to the "latch" behavior observed in intact muscle. ADP in the vicinity of the contractile proteins, thus, might have a role in modulating the cross-bridge turnover primarily in the slow and economical smooth muscle types. In the living muscle, force can be supported by unphosphorylated cross-bridges, and it should be noted that our experiments were performed in maximally phosphorylated muscles. However, biochemical data (Greene and Sellers, 1987) and studies on smooth muscle in rigor (Arheden and Arner, 1992) do not suggest a large effect of myosin light chain phosphorylation on the ADP binding to the A-M state. The slow muscle is less affected by increased inorganic phosphate. Although the effects of phosphate occur at comparatively high concentrations, the lower phosphate sensitivity could be another mechanism, in addition to ADP binding, for the slow muscle to maintain tone during sustained contractions and in situations with impaired energy supply.

We are grateful for the excellent technical assistance of Mrs. Christina Persson. We also thank Dr. Juris Galvanovskis for the help with solving mathematical problems associated with the nonlinear elastic model.

This work was supported by grants from the Swedish Medical Research Council (No. 04X-12584 to U. Malmqvist and No. 04X-8268 to A. Arner) and the Medical Faculty Lund University.

Submitted: 4 December 2000

Revised: 6 March 2001

Accepted: 27 March 2001

REFERENCES

- Arheden, H., and A. Arner. 1992. Effects of magnesium pyrophosphate on mechanical properties of skinned smooth muscle from the guinea pig taenia coli. *Biophys. J.* 61:1480-1494.
- Arheden, H., A. Arner, and P. Hellstrand. 1988. Cross-bridge behaviour in skinned smooth muscle of the guinea-pig taenia coli at altered ionic strength. *J. Physiol.* 403:539-558.
- Arner, A. 1982. Mechanical characteristics of chemically skinned guinea-pig taenia coli. *Pflügers Arch.* 395:277-284.
- Arner, A. 1983. Force-velocity relation in chemically skinned rat portal vein. Effects of Ca^{2+} and Mg^{2+} . *Pflügers Arch.* 397:6-12.
- Arner, A., and P. Hellstrand. 1985. Effects of calcium and substrate on force-velocity relation and energy turnover in skinned smooth muscle of the guinea-pig. *J. Physiol.* 360:347-365.
- Arner, A., P. Hellstrand, and J.C. Rüegg. 1987. Influence of ATP, ADP and AMPPNP on the energetics of contraction in skinned smooth muscle. *Prog. Clin. Biol. Res.* 245:43-57.
- Bozler, E. 1941. Action potentials and conduction of excitation in muscle. *Biol. Symp.* 3:95-109.
- Conibear, P.B., and M.A. Geeves. 1998. Cooperativity between the two heads of rabbit skeletal muscle heavy meromyosin in binding to actin. *Biophys. J.* 75:926-937.
- Cooke, R., and E. Pate. 1985. The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys. J.* 48:789-798.
- Cremonesi, C.R., and M.A. Geeves. 1998. Interaction of actin and ADP with the head domain of smooth muscle myosin: implications for strain-dependent ADP release in smooth muscle. *Biochemistry.* 37: 1969-1978.
- Dantzig, J.A., R.J. Barsotti, S. Manz, H.L. Sweeney, and Y.E. Goldman. 1999. The ADP release step of the smooth muscle cross-bridge cycle is not directly associated with force generation. *Biophys. J.* 77:386-397.
- Fabiato, A. 1981. Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cell from the adult rat or rabbit ventricle. *J. Gen. Physiol.* 78:457-497.
- Fabiato, A., and F. Fabiato. 1979. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J. Physiol.* 75:463-505.
- Feldhaus, P., T. Frohlich, R.S. Goody, M. Isakov, and R.H. Schirmer. 1975. Synthetic inhibitors of adenylate kinases in the assays for ATPases and phosphokinases. *Eur. J. Biochem.* 57:197-204.
- Fisher, M.J., and P.F. Dillon. 1988. Direct determination of ADP in hypoxic porcine carotid artery using ^{31}P NMR. *NMR Biomed.* 1:121-126.
- Fletcher, R., and M. Powell. 1963. A rapidly convergent descent method for minimization. *Comp. J.* 6:163-168.
- Fuglsang, A., A. Khromov, K. Torok, A.V. Somlyo, and A.P. Somlyo. 1993. Flash photolysis studies of relaxation and cross-bridge detachment: higher sensitivity of tonic than phasic smooth muscle to MgADP. *J. Muscle Res. Cell Motil.* 14:666-677.

- Gollub, J., C.R. Cremona, and R. Cooke. 1996. ADP release produces a rotation of the neck region of smooth myosin but not skeletal myosin. *Nat. Struct. Biol.* 3:796–802.
- Greene, L.E., and J.R. Sellers. 1987. Effect of phosphorylation on the binding of smooth muscle heavy meromyosin X ADP to actin. *J. Biol. Chem.* 262:4177–4181.
- Hellstrand, P., and R.J. Paul. 1983. Phosphagen content, breakdown during contraction, and O₂ consumption in rat portal vein. *Am. J. Physiol.* 244:C250–C258.
- Hellstrand, P., and H.J. Vogel. 1985. Phosphagens and intracellular pH in intact rabbit smooth muscle studied by 31P-NMR. *Am. J. Physiol.* 248:C320–C329.
- Hibberd, M.G., J.A. Dantzig, D.R. Trentham, and Y.E. Goldman. 1985. Phosphate release and force generation in skeletal muscle fibers. *Science.* 228:1317–1319.
- Hill, A.V. 1938. The heat of shortening and the dynamic constants of shortening. *Proc. R. Soc.* 126:231–252.
- Horiuti, K., A.V. Somlyo, Y.E. Goldman, and A.P. Somlyo. 1989. Kinetics of contraction initiated by flash photolysis of caged adenosine triphosphate in tonic and phasic smooth muscles. *J. Gen. Physiol.* 94:769–781.
- Huang, Q.Q., S.A. Fisher, and F.V. Brozovich. 1999. Forced expression of essential myosin light chain isoforms demonstrates their role in smooth muscle force production. *J. Biol. Chem.* 274:35095–35098.
- Jaworowski, A., N. Öztürk, and A. Arner. 1999. Inhibition of force and shortening in smooth muscle by vanadate. *Pflügers Arch.* 438:224–231.
- Kelley, C.A., J.R. Sellers, P.K. Goldsmith, and R.S. Adelstein. 1992. Smooth muscle myosin is composed of homodimeric heavy chains. *J. Biol. Chem.* 267:2127–2130.
- Khromov, A., A.P. Somlyo, and A.V. Somlyo. 2001. Photolytic release of MgADP reduces rigor force in smooth muscle. *Biophys. J.* 80:1905–1914.
- Khromov, A.S., A.V. Somlyo, and A.P. Somlyo. 1996. Nucleotide binding by actomyosin as a determinant of relaxation kinetics of rabbit phasic and tonic smooth muscle. *J. Physiol.* 492:669–673.
- Khromov, A., A.V. Somlyo, and A.P. Somlyo. 2000. MgADP binding to cross bridges reduces rigor force in smooth muscle. *Biophys. J.* 78:109A.
- Krisanda, J.M., and R.J. Paul. 1983. Phosphagen and metabolite content during contraction in porcine carotid artery. *Am. J. Physiol.* 244:C385–C390.
- Lauzon, A.-M., M.J. Tyska, A.S. Rovner, Y. Freyzon, D.M. Warshaw, and K.M. Trybus. 1998. A 7-amino-acid insert in the heavy chain nucleotide binding loop alters the kinetics of smooth muscle myosin in the laser trap. *J. Muscle Res. Cell Motil.* 19:825–837.
- Malmqvist, U., and A. Arner. 1991. Correlation between isoform composition of the 17 kDa myosin light chain and maximal shortening velocity in smooth muscle. *Pflügers Arch.* 418:523–530.
- Malmqvist, U., M. Löfgren, and A. Arner. 1999. Effects of ATP, ADP and inorganic phosphate on force and shortening velocity differ between fast and slow smooth muscles. *Biophys. J.* 76:A284.
- Mannherz, H.G. 1968. ATP-cleavage and ATP-diffusion in oscillating muscle fibers. *Pflügers Arch.* 303:230–248.
- Marston, S.B., and E.W. Taylor. 1980. Comparison of the myosin and actomyosin ATPase mechanisms of the four types of vertebrate muscles. *J. Mol. Biol.* 139:573–600.
- Matthew, J.D., A.S. Khromov, K.M. Trybus, A.P. Somlyo, and A.V. Somlyo. 1998. Myosin essential light chain isoforms modulate the velocity of shortening propelled by nonphosphorylated cross-bridges. *J. Biol. Chem.* 273:31289–31296.
- Nishiye, E., A.V. Somlyo, K. Torok, and A.P. Somlyo. 1993. The effects of MgADP on cross-bridge kinetics: a laser flash photolysis study of guinea-pig smooth muscle. *J. Physiol.* 460:247–271.
- Österman, A., and A. Arner. 1995. Effects of inorganic phosphate on cross-bridge kinetics at different activation levels in skinned guinea-pig smooth muscle. *J. Physiol.* 484:369–383.
- Pate, E., and R. Cooke. 1989. A model of crossbridge action: the effects of ATP, ADP and Pi. *J. Muscle Res. Cell Motil.* 10:181–196.
- Pate, E., M. Lin, K. Franks-Skiba, and R. Cooke. 1992. Contraction of glycerinated rabbit slow-twitch muscle fibers as a function of MgATP concentration. *Am. J. Physiol.* 262:C1039–C1046.
- Rosenfeld, S.S., J. Xing, M. Whitaker, H.C. Cheung, F. Brown, A. Wells, R.A. Milligan, and H.L. Sweeney. 2000. Kinetic and spectroscopic evidence for three actomyosin:ADP states in smooth muscle. *J. Biol. Chem.* 275:25418–25426.
- Rovner, A.S., Y. Freyzon, and K.M. Trybus. 1997. An insert in the motor domain determines the functional properties of expressed smooth muscle myosin isoforms. *J. Muscle Res. Cell Motil.* 18:103–110.
- Schoenberg, M., and E. Eisenberg. 1987. ADP binding to myosin cross-bridges and its effect on the cross-bridge detachment rate constants. *J. Gen. Physiol.* 89:905–920.
- Siemankowski, R.F., M.O. Wiseman, and H.D. White. 1985. ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc. Natl. Acad. Sci. USA.* 82:658–662.
- Sjuve, R., H. Haase, I. Morano, B. Uvelius, and A. Arner. 1996. Contraction kinetics and myosin isoform composition in smooth muscle from hypertrophied rat urinary bladder. *J. Cell Biochem.* 63:86–93.
- Somlyo, A.P., and A.V. Somlyo. 1968. Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. *Pharmacol. Rev.* 20:197–272.
- Somlyo, A.V., Y.E. Goldman, T. Fujimori, M. Bond, D.R. Trentham, and A.P. Somlyo. 1988. Cross-bridge kinetics, cooperativity, and negatively strained cross-bridges in vertebrate smooth muscle. A laser-flash photolysis study. *J. Gen. Physiol.* 91:165–192.
- Sweeney, H.L., S.S. Rosenfeld, F. Brown, L. Faust, J. Smith, J. Xing, L.A. Stein, and J.R. Sellers. 1998. Kinetic tuning of myosin via a flexible loop adjacent to the nucleotide binding pocket. *J. Biol. Chem.* 273:6262–6270.
- Warshaw, D.M., J.M. Desrosiers, S.S. Work, and K.M. Trybus. 1991. Effects of MgATP, MgADP, and Pi on actin movement by smooth muscle myosin. *J. Biol. Chem.* 266:24339–24343.
- White, S., A.F. Martin, and M. Periasamy. 1993. Identification of a novel smooth muscle myosin heavy chain cDNA: isoform diversity in the S1 head region. *Am. J. Physiol.* 264:C1252–C1258.
- Whittaker, M., E.M. Wilson-Kubalek, J.E. Smith, L. Faust, R.A. Milligan, and H.L. Sweeney. 1995. A 35-A movement of smooth muscle myosin on ADP release. *Nature.* 378:748–751.