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Myosin light chain phosphorylation and the cross-bridge cycle at low substrate concentration in chemically skinned guinea pig *Taenia coli*

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Abstract. Force-velocity relations, rate of ATP turnover (J_{ATP}), and phosphorylation of the 20,000 D myosin light chains (LC_{20}) were measured in chemically skinned guinea pig *Taenia coli*. Relative LC_{20} phosphorylation at 3.2 mM MgATP was 17% in relaxed tissues at pCa 9, and increased with force at increasing $[Ca^{2+}]$ to a maximum of 67% at pCa 4.5. Force at pCa 4.5 was dependent on the MgATP concentration with a half-maximal response at about 0.1 mM. At 0.1 mM MgATP LC_{20} phosphorylation at pCa 4.5 was 38%. Both J_{ATP} and the maximal shortening velocity (V_{max}) were reduced in 0.1 mM MgATP, to 32% and 43%, respectively, of their values at 3.2 mM MgATP. Low-MgATP thus inhibits both LC_{20} phosphorylation and the extent and rate of cross-bridge interaction. High levels of LC_{20} phosphorylation, independent of Ca^{2+} and MgATP concentrations, were obtained by treatment with ATP-γ-S. Maximal force at 3.2 mM MgATP after LC_{20} thiophosphorylation was unchanged, whereas halfmaximal force occurred at 0.065 mM MgATP after thiophosphorylation, compared to 0.13 mM after activation by Ca^{2+}. The contraction in thiophosphorylated preparations at low-MgATP (0.1 mM) was associated with submaximal V_{max} (60%) and J_{ATP} (27%). The results show that LC_{20} phosphorylation is correlated with the degree of force development in the Ca^{2+} activated contraction, both when Ca^{2+} and MgATP concentrations are varied. The reduced force and rate of crossbridge turnover in lowMgATP are however primarily mediated by an influence of MgATP on the cross-bridge cycle, which is separate from the effect on LC_{20} phosphorylation.

Key words: Smooth muscle – ATP – ATP-γ-S – Ca^{2+} – Force-velocity relation – Energetics

Introduction

Contraction in smooth muscle is thought to be governed by a regulating system involving Ca^{2+} and phosphorylation of the 20,000 D light chains (LC_{20}) of the myosin molecule, although the details of this regulation are still under debate. Of considerable interest for the characterization of the organized smooth muscle contractile system are the properties of the cross-bridge cycle under defined regulatory conditions. Experiments on chemically skinned smooth muscle preparations have shown that $[Ca^{2+}]$ influences not only the force development but also the maximal shortening velocity (V_{max}) and the rate of ATP turnover (J_{ATP}), both absolutely and in relation to developed force (Arner 1982, 1983; Arner and Hellstrand 1983, 1985; Paul et al. 1983; Güth and Mrwa 1982). These findings are consistent with the view that increased $[Ca^{2+}]$ increases both the number of attached cross-bridges and their specific rate of turnover. In the converse situation, activation at saturating $[Ca^{2+}]$ at different levels of [MgATP], it was found that both force, V_{max} and the energetic cost of force maintenance were reduced at low [MgATP] (Arner and Hellstrand 1985). It should be recognized that LC_{20} phosphorylation is in itself an ATP-dependent process and hence it is a priori possible that LC_{20} phosphorylation might provide a link between cross-bridge properties and [MgATP] as well. To resolve this question information is needed on the level of LC_{20} phosphorylation at different $[Ca^{2+}]$ and [MgATP] with accompanying determinations of mechanical and energetic indices of cross-bridge activity. This approach has been followed in the present study on chemically skinned guinea pig *Taenia coli* smooth muscle. The ATP utilization during isometric contraction was measured in parallel with determinations of the force-velocity relation and the level of LC_{20} phosphorylation. The effects of differing $[Ca^{2+}]$ and [MgATP] were investigated. The ATP-analogue ATP-γ-S, which causes LC_{20} thiophosphorylation resistant to phosphatase activity (Sherry et al. 1978; Cassidy et al. 1979) was utilized in order to separate effects of MgATP on the phosphorylation step and on the cross-bridge interaction in itself.

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Methods

Preparation and solutions. Guinea pig *Taenia coli* preparations were dissected out, chemically skinned by exposure to a medium containing 1% Triton X-100 and stored at −15° C as described earlier (Arner and Hellstrand 1985). Strips with a length of 4–7 mm and wet wt. of 0.3–1.3 mg were cut out and mounted as described below. In experiments where myosin light chain phosphorylation was determined, slightly thicker preparations were used (approximate length 10 mm, weight 4 mg). The muscle length was measured using a microscope with an ocular scale. The wet weight was determined on a Cahn electrobalance after gently blotting the preparation between two sheets of filter paper. The cross-sectional area was calculated from muscle wet weight and length assuming a density of 1.05 mg × mm^{-3}. The muscle strips were incubated in solutions containing (mM): N-tris-(hydroxymethyl)-methyl-2-aminomethane sulfonic acid

(TES) 30, dithioerythritol 0.5, phosphoenolpyruvate (PEP) 5, sodium azide 1, and pyruvate kinase 20 U/ml. Varied MgATP concentrations were obtained by adding Na_2ATP and $MgCl_2$. The concentration of free-Mg^{2+} was held constant at 2 mM. The ionic strength was adjusted to 0.15 with KCl and pH to 6.9 with KOH. Calmodulin in a concentration of 1 μM was present in all solutions. The Ca^{2+}-concentration of the "relaxing" solution used for the initial incubation and for relaxation later in the experiments was 10^{-9} M, obtained by adding appropriate amounts of K_2-ethyleneglycol-tetraacetic acid (K_2-EGTA) and K_2Ca-EGTA while keeping the total EGTA concentration at 4 mM. The "activating" solution had a similar composition except that the Ca^{2+}-concentration was $10^{-4.5}$ M. Media of intermediate [Ca^{2+}] were obtained by mixing the two solutions. Thiophosphorylation was achieved by incubating the strips in activating medium containing 2 mM ATP-γ-S, but no ATP or ATP-regenerating system, to prevent formation of ATP from ADP contaminating the ATP-γ-S, or released during thiophosphorylation. All experiments were carried out at room temperature (21–22° C). The free ion concentrations and ionic strength of the media were computed as described by Fabiato and Fabiato (1979), using the equilibrium constants given by Fabiato (1981). ATP-γ-S was obtained from Boehringer Mannheim, FRG, all other chemicals from Sigma Chemical Co., St. Louis, MO, USA. Calmodulin was a gift from Eva Thulin, Division of Physical Chemistry 2, Chemical Centre, University of Lund.

Isometric experiments. Isometric experiments were carried out with the strips tied at one end to fixed glass rods and at the other connected to Grass FTO3 force transducers mounted on adjustable stands. The strips were immersed in 0.3 ml solution contained in plastic cups mounted on vertical shafts rotating at 0.5 Hz to provide mixing of the solution. Passive force was initially adjusted to 3 mN in relaxing medium (pCa = $-\log$ [Ca^{2+}] = 9) with 3.2 mM MgATP. During the following 10–15 min in this solution passive force decayed to about 1 mN at unchanged muscle length. For the strip dimensions used this length was found to be approximately that giving maximal active force, and was designated as L_0. The strip was activated at this length. Active force was measured at plateau of the contractions. For measurement of ATP turnover the incubation cups were exchanged at 10 min intervals and frozen at −80° C together with solution blanks. ATP breakdown was measured using an NADH-linked fluorimetric assay of pyruvate released from PEP in the incubation medium (Peterson 1980; Arner and Hellstrand 1985).

Quick release experiments. Isotonic quick release experiments for determination of force-velocity relations were performed using a lever system (Hellstrand and Johansson 1979), with a protocol similar to that described by Arner and Hellstrand (1985). The experiments were carried out at a passive of 0.1–0.2 mN (muscle length about 0.94 L_0). The sequence of force-velocity determinations was as follows. First, one force-velocity relation was obtained at pCa 4.5, 3.2 mM MgATP. The strip was then relaxed and after about 15 min activated again in pCa 4.5, 0.1 mM MgATP and a new force-velocity relation obtained. After relaxation the strip was transferred to ATP-free medium and then to 2 mM ATP-γ-S for 10 min to achieve thiophosphorylation (see Results). It was then incubated, first in pCa 9, 0.1 mM MgATP, then in pCa 9, 3.2 mM MgATP, and finally in pCa 4.5, 3.2 mM MgATP. The strip was transferred directly between these three solutions and a force-velocity relation determined in each. We have previously showed that the shortening velocity at each afterload is a function of time after release (Arner and Hellstrand 1985). In the present study we therefore evaluated the shortening velocity at a fixed point in time (100 ms) after release. Force-velocity relations were fitted by the Hill (1938) equation: $V = b\,(1 - P/P_0)/(P/P_0 + a/P_0)$ where all force values (P) were normalized to the isometric force before release (P_0). V is shortening velocity and a and b are constants. The curve-fitting was done using a nonlinear least-squares method (Fletcher and Powell 1963) to minimize the perpendicular distance from the data points to the fitted curve.

Determination of myosin light chain phosphorylation. The level of myosin light chain phosphorylation was determined in *Taenia* strips that were relaxed or activated in media of different [Ca^{2+}] and [MgATP]. After incubation under isometric conditions as described above the strips were clamped between stainless steel blocks precooled in liquid N_2. The frozen strips were stored at −80° C for a maximum of 2 weeks. For extraction, they were transferred to glass homogenizers cooled in liquid N_2. The muscles were pulverized and mixed with 200 μl 15% trichloroacetic acid (TCA) which rapidly froze at the bottom of the homogenizer. The homogenizer was transferred to ice and the sample was homogenized in the thawing TCA. The sample was diluted with 100 μl TCA and centrifuged at 18,000 × *g* for 2 min. The precipitate was washed with cold diethylether and dissolved in 0.025 ml/mg tissue wet wt. of a solution containing: 1% sodium laurylsulphate (SDS), 10% glycerol and 20 mM dithiothreitol (DTT). Myosin light chain phosphorylation was determined as described by Driska et al. (1981) with two-dimensional electrophoresis. The isoelectric focusing gels (diameter 3 mm, length 13 cm) contained 2% Pharmacia 6.5–4.0 ampholytes, 9 M urea and 2% Nonidet-P-40. Each gel was loaded with 100 μl of muscle extract. Isoelectric focusing was performed with cooling (about 17° C) for 18 h at 400 V an for 1 h at 800 V with 20 mM NaOH as anode- and 10 mM H_3PO_4 as cathode-solution. The gels were directly, or after a maximum of 1 week at −15° C, loaded on the second dimension gels. The separating gels (7 cm long, 1.4 mm thick) contained: 13% polyacrylamide; 0.1% SDS in 0.375 M Tris-HCl (pH 8.8). The gels were stained with Coomassie blue and scanned in the 20,000 D region in the isoelectric focusing direction with a Zeineh soft laser scanning densitometer (Biomed Instruments, Chicago, USA). Standard proteins were used to determine the pH gradient (Pharmacia pI markers) and the molecular weight calibration (Sigma molecular weight markers).

For determination of protein content muscle strips were frozen as described above, either fresh after dissection and incubation for 2 h in physiological Krebs medium, or after skinning and storage in glycerol at −15° C. The strips were extracted in 1 M NaOH and protein determination performed by the Lowry method. For control of possible changes in tissue weight a set of *Taenia* strips were weighed before and after skinning. The results indicated no change in tissue weight as a result of the skinning and storage.

Statistical analysis. Values are given as means ± SEM, with the number of observations within parentheses. Unless

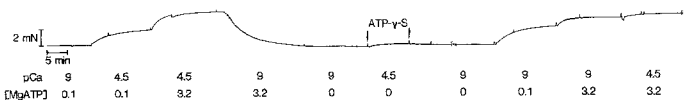

Fig. 1. Contraction of chemically skinned guinea-pig *Taenia coli* on activation by Ca^{2+} (first) and in the absence of Ca^{2+} after LC_{20} thiophosphorylation (second). Concentrations of Ca^{2+} (pCa $= -\log [Ca^{2+}]$) and MgATP (mM) indicated below record. 2 mM ATP-γ-S present between *arrows*. Artifacts on force record show exchange of incubation cups for measurement of ATP breakdown

stated otherwise, values of force, shortening velocities, or ATP turnover, are reported relative to control values in the individual strips obtained under incubation in "activating" solution as defined above. Statistical comparisons were made with the Student's *t*-test for unpaired or paired data as appropiate.

Results

Effects of [MgATP] and LC_{20} thiophosphorylation on isometric force

The response of a skinned *Taenia* preparation to the different activation conditions used in this investigation is exemplified by the recording shown in Fig. 1. The muscle strip is first incubated in a medium of low Ca^{2+} concentration (pCa 9) containing 0.1 mM MgATP. Exposure to a high $[Ca^{2+}]$ (pCa 4.5) causes contraction amounting to 45% of the maximal force level reached on subsequent exposure to an optimal [MgATP] of 3.2 mM. After relexation in pCa 9 the strip is kept in an ATP-free solution for 15 min before being exposed to 2 mM ATP-γ-S in pCa 4.5 for 10 min. Under these conditions there is only minimal force production as shown in the figure. Subsequent exposure to 0.1 and 3.2 mM MgATP in the absence of Ca^{2+} (pCa 9) results in force development to 62 and 90%, respectively, of the control response. Addition of Ca^{2+} (pCa 4.5) causes a small additional force increase back to the control level. Subsequent removal of ATP results in a sluggish and incomplete relaxation (not shown).

To characterize the force response to [MgATP] a series of experiments was carried out where *Taenia* strips were activated in pCa 4.5 at a certain [MgATP] until a plateau force had developed (15 min) and then in 3.2 mM MgATP, still at pCa 4.5, to determine the maximal response. The force at the lower [MgATP] was expressed as a percentage of the maximal response. Parallel experiments were carried out on untreated and thiophosphorylated strips. The results are as shown in Fig. 2. In the untreated preparations force is dependent on [MgATP] with a concentration giving 50% response (EC_{50}) of 0.13 mM. After thiophosphorylation, force development at intermediate [MgATP] is increased and the EC_{50} is lowered by a factor of 2, to 0.065 mM. The maximal response is however unchanged by the thiophosphorylation, as shown by Fig. 1 (see also Fig. 3 below).

ATP turnover

Samples for measurement of the rate of ATP turnover (J_{ATP}) were taken as shown in Fig. 1, where the artifacts on the force record indicate exchange of incubation cups. The results are shown in Fig. 3. In the relaxed state before thiophosphorylation (A) J_{ATP} is negligible but on activation in 0.1 mM

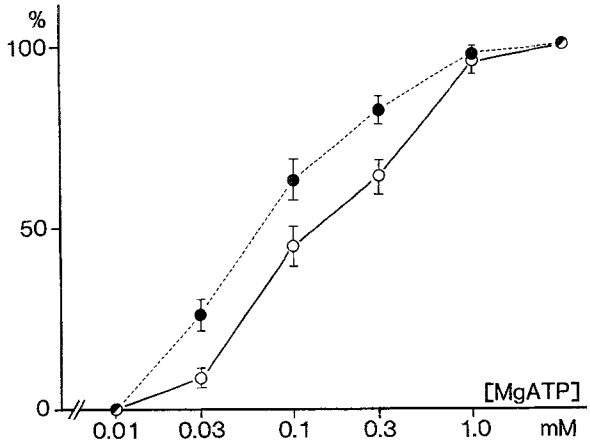

Fig. 2. Force response in different MgATP concentrations to activation by Ca^{2+} (○, pCa 4.5) or LC_{20} thiophosphorylation (●). Each strip (Ca^{2+}-activated or thiophosphorylated) was exposed to one test concentration of MgATP and then to 3.2 mM to give a maximal (= 100%) contraction ($n = 6$ in each point)

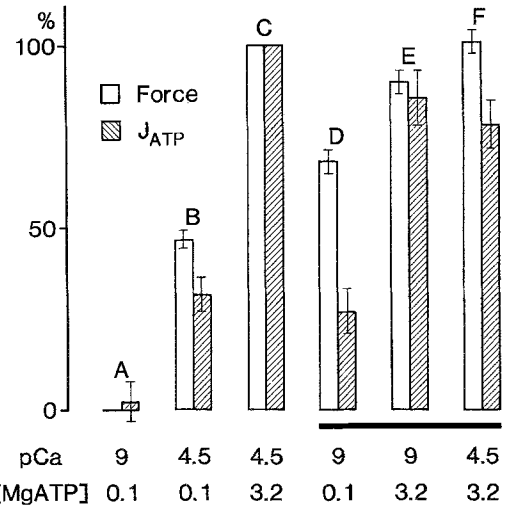

Fig. 3. Force and ATP breakdown (J_{ATP}) during contractions as illustrated in Fig. 1. Values are shown normalized to the Ca^{2+}-activated contraction of the individual muscle strips at pCa 4.5 in 3.2 mM MgATP (*C*). *Horizontal bar* indicates values obtained after thiophosphorylation (*D*–*F*). Force and J_{ATP} corresponding to 100% were, respectively, 30 ± 6 mN/mm² and 0.73 ± 0.06 μmol/min × g wet wt. ($n = 6$)

MgATP (B) it rises in approximate proportion to the force when normalized to the maximal response (C). After thiophosphorylation (D–F, indicated by horizontal bar in Fig. 3) J_{ATP} relative to force is somewhat lower. Particularly at 0.1 mM MgATP (D) there is a discrepancy between relative force and J_{ATP} which is not seen before thiophosphorylation (B). This is not caused by the difference in $[Ca^{2+}]$, which was verified in a separate series of experiments (data not shown). Moreover, there is no significant difference in J_{ATP} between E and F, where $[Ca^{2+}]$ is altered at optimal [MgATP] after thiophosphorylation.

Force-velocity relations

Force-velocity relations were determined using an essentially similar protocol as in the isometric experiments for J_{ATP}

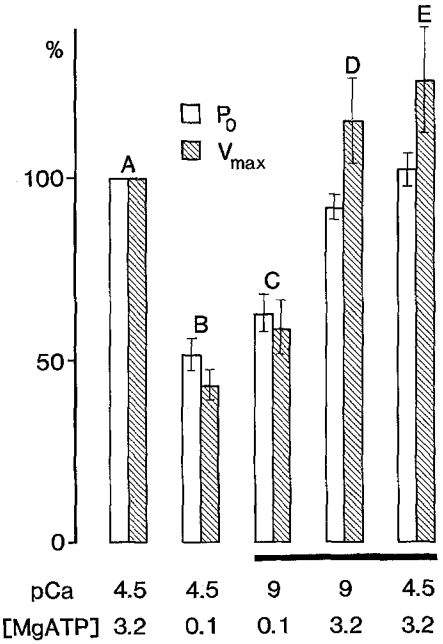

Fig. 4. Isometric force (P_0) and maximal shortening velocity (V_{max}) during Ca^{2+}-activation (*A, B*) and after LC_{20} thiophosphorylation (*C–E, horizontal bar*). Values in column *A* represent 100% for each muscle strip ($n = 8$): $P_0 = 51 \pm 8$ mN/mm^2, $V_{max} = 0.11 \pm 0.01$ L/s

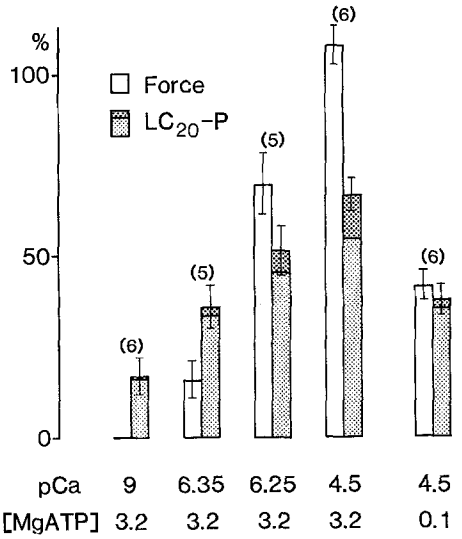

Fig. 5. Force and relative LC_{20} phosphorylation (LC_{20}-P) at different Ca^{2+} and MgATP concentrations. Force of each muscle strip expressed as a percentage of force in a reference contracture at pCa 4.5, 3.2 mM MgATP. LC_{20}-P shows amount of phosphorylated light chains as percentage of total light chain content. The upper, coarsely dotted parts of the columns show measurements including satellite bands. Number of strips indicated within parentheses above each column

measurements (Fig. 1), except that the preparations were relaxed between the Ca^{2+}-activations to reduce the time of continuous contraction, and that the exposure to pCa 4.5, 3.2 mM MgATP, was done before that to 0.1 mM MgATP. This allowed responses to 0.1 mM MgATP to be determined in sequence before and after thiophosphorylation.

About eight releases to a range of different afterloads were performed at the plateau of each contraction within a time span of 15–20 min. The muscle was restretched to the original length after each release and allowed to recover a steady force before the next release was done. Under these conditions the active force level is well maintained as shown e.g. by original records of similar experiments published earlier (Arner 1982). The force-velocity relations were fitted by the Hill (1938) equation. The force levels (P_0) and maximal shortening velocities (V_{max}) are shown in Fig. 4. There is a parallel variation of P_0 and V_{max} under the different conditions tested. The force-velocity relations D and E, determined at 3.2 mM MgATP after thiophosphorylation, differ only slightly from the control (A) in isomeric force P_0. However, V_{max} in E, but not in D, is significantly ($P < 0.05$) larger than the control value (A). The parameter a/P_0 of the fitted Hill equation is independent of treatment (range 0.22–0.26), whereas b consequently varies in parallel with V_{max} ($V_{max} = b \times P_0/a$).

Myosin LC_{20} phosphorylation

Chemically skinned preparations, treated with media of different [Ca^{2+}] and [MgATP], were analysed for the level of myosin light chain phosphorylation by two-dimensional electrophoresis as described in Methods. The phosphorylated and unphosphorylated light chains were located in the Mw 20,000 region at approximate pI of 4.9 and 5.0 respectively. One, and occasionally two, satellite spots with lower pI (cf. Driska et al. 1981) were observed under conditions with high degree of phosphorylation. Whether the satellite light chain spots reflect alternative location or extent of phosphorylation, isoforms or artifactual protein modification during the electrophoresis is at present unclear (Ledvora et al. 1983; Gagelman et al. 1984; Haeberle et al. 1984). Since the satellite spots reflect phosphorylated proteins (Gagelmann et al. 1984) they are in the present study included in the calculation of the degree of phosphorylation. Figure 5 shows that the relative degree of phosphorylation (dotted bars; upper areas show the extent of satellite spots) increase together with force (open bars) at increasing [Ca^{2+}] at 3.2 mM MgATP. Preparations activated with pCa 4.5 at 0.1 mM MgATP showed a reduced level of phosphorylation as well as a reduced force compared to the values at maximal activation (pCa 4.5, 3.2 mM MgATP). The values in 0.1 mM MgATP fall close to the relation between force and phosphorylation at varied [Ca^{2+}] in optimal [MgATP]. The possible influence of ATP diffusion limitation in low-MgATP was considered. When the thicker preparations used in the phosphorylation measurements were compared to preparations cut down to the same size as that used in quick release and ATP turnover experiments, we found similar reduction in force in 0.1 mM MgATP. Moreover, in four thin preparations, freeze-clamped in pCa 4.5, 0.1 mM MgATP, the relative degree of phosphorylation (41.3%) was similar to that given in Fig. 5.

In two preparations freeze-clamped in 3.2 mM MgATP after thiophosphorylation, high degrees of phosphorylation were observed in pCa 9 (75%) and in pCa 4.5 (91.2%).

The protein content of chemically skinned preparations was 52% ($n = 5$, $P < 0.05$) of that of intact *Taenia* when compared on the basis of wet wt. (unchanged by skinning). When intact and skinned preparations of similar weights were extracted and subjected to the two-dimensional

electrophoresis, intact preparations showed a higher amount of light chains. These results showing protein extraction during chemical skinning also suggest that myosin light chains may be extracted to some extent.

Discussion

Activation of contraction in the skinned *Taenia coli* by increasing the Ca^{2+} concentration is accompanied by increasing level of LC_{20}, phosphorylation. This is in agreement with previous results on skinned smooth muscle (Hoar et al. 1979; Cassidy et al. 1979; Gagelmann and Güth 1985). Exposure to ATP-γ-S results in an almost maximal level of LC_{20} thiophosphorylation (cf. also Hoar et al. 1979). The range of LC_{20} phosphorylation found in untreated preparations was from 17% in relaxed to 67% in maximally activated tissues. Since LC_{20} phosphorylation represents a steady state situation depending on the kinase and phosphatase activity the theoretical maximum of 100% is not reached during contraction (cf. Peterson 1982). However, 67% apparently is sufficient for essentially maximal activation since there was little further tension development after thiophosphorylation. It has been shown (Hoar et al. 1979; Cassidy et al. 1979) that smooth muscle LC_{20} thiophosphorylated by radioactively labelled [^{35}S]ATP-γ-S retain their ^{35}S-labelling after addition of cold MgATP, and that after incubation of thiophosphorylated strips with [γ-^{32}P]ATP no ^{32}P incorporation in the LC_{20} region occurs. Hence LC_{20} thiophosphorylation levels are not expected to be influenced by the different MgATP concentrations used in the present experiments. This is further supported by the essentially maximal force development on addition of 3.2 mM MgATP, pCa 9, after a period of submaximal contraction at 0.1 mM MgATP, pCa 9 (Fig. 1). Thiophosphorylation in the absence of MgATP results in negligible force development (Fig. 1). This confirms that ATP-γ-S is not a substrate for continuing cross-bridge activity.

The relation between J_{ATP} and developed force (energetic tension cost) and the maximal shortening velocity (V_{max}) provide information on the rate of cross-bridge turnover (see e.g. Rüegg 1971; Hellstrand and Paul 1982). It is possible that other enzymatic systems than the contractile proteins could contribute to J_{ATP} in the Ca^{2+} activated untreated strips. Little is known about which cellular functions are actually preserved in the skinned preparations. There is a considerable loss of protein as estimated from measurements of total protein content in the present study. Myosin LC_{20} stained less intensely after skinning than in intact preparations when referred to tissue wet weight. This indicates that a large measure of protein extraction occurs, which also affects the contractile proteins and probably accounts for the lower force per cross-sectional area after skinning. In the thiophosphorylated preparations it is possible to vary [Ca^{2+}] without altering force. The addition of Ca^{2+} to contracted thiophosphorylated strips, both at optimal and low [MgATP], did not cause an increase in J_{ATP} (Fig. 3). Hence it is not likely that the energetic tension cost has increased either. Unless the tension cost is actually decreased, the result rules out the activation by Ca^{2+} of ATPase activity unrelated to tension generation. The comparison of J_{ATP} in contracted untreated and thiophosphorylated states is more complex. Our results (Fig. 3) suggest that J_{ATP} in relation to force after thiophosphorylation is actually decreased, particularly at 0.1 mM MgATP. One cause of this effect could be the presence of continuing protein phosphorylation-dephosphorylation cycles in the untreated preparation, which are turned off by irreversible thiophosphorylation after exposure to ATP-γ-S. With respect to LC_{20} phosphorylation the expected influence on overall J_{ATP} would be small, as suggested by calculations presented by Peterson (1982), and would not account for all of the observed effect. However, the actual rate of LC_{20} phosphorylation deserves further study, including direct measurements.

The lack of an effect of Ca^{2+} on J_{ATP} after thiophosphorylation may be considered in relation to studies on actin-activated ATPase activity of thiophosphorylated smooth muscle myosin. Using chicken gizzard, Sherry et al. (1978) reported no effect of Ca^{2+} on the fully phosphorylated myosin free of kinase and phosphatase activities. Heaslip and Chacko (1985), on the other hand, reported a potentiation of the ATPase activity by Ca^{2+}, which was however absent at concentrations of free Mg^{2+} ions exceeding 5 mM, which were the conditions used by Sherry et al. (1978). The free Mg^{2+} concentration in our experiments was 2 mM, and hence an effect analogous to that reported by Heaslip and Chacko (1985) should have been detectable. Further studies are needed to elucidate this point, which has a direct bearing on the possibility that Ca^{2+} participates in contractile regulation via more than one mechanism.

At 0.1 mM MgATP and pCa 4.5 the phosphorylation level is 40% lower than at 3.2 mM (Fig. 5). This is consistent with the K_m values (0.05 and 0.1 mM) for myosin light chain kinase in solution reported by Adelstein and Klee (1981) and by DiSalvo et al. (1981) in gizzard and arterial smooth muscle, respectively. A reduction of the phosphorylation rate at low-[MgATP] does however not affect the relation between relative force and [Ca^{2+}], as shown by results presented earlier (Arner and Hellstrand 1985). The concentration-response relationship between MgATP and isometric force (Fig. 2) is affected by thiophosphorylation, which lowers EC_{50} by a factor of about 2. There is still however a considerable MgATP requirement which is clearly greater than that reported in skinned skeletal muscle (Cooke and Bialek 1979; Ferenczi et al. 1984). The present results thus show that a high MgATP requirement for force development in the smooth muscle is an intrinsic property of the cross-bridge system in this tissue, and not primarily a consequence of the MgATP sensitivity of LC_{20} phosphorylation.

The relationship of V_{max} to [MgATP] resembles that of force (Fig. 4). Both are reduced to a similar extent at 0.1 mM MgATP whether or not the *Taenia* strip has been thiophosphorylated. In the untreated strip at pCa 4.5 LC_{20} phosphorylation at 0.1 mM MgATP is clearly lower than at 3.2 mM (Fig. 5), whereas as we have seen, after thiophosphorylation it is essentially maximal even at pCa 9. At 3.2 mM MgATP, both at pCa 9 and 4.5, V_{max} after thiophosphorylation tends to be higher when compared to the untreated maximally activated state (Fig. 4). In skinned *Taenia* that had not been thiophosphorylated we were not able to demonstrate an effect on V_{max} separate from that on force when Ca^{2+} was varied (Arner and Hellstrand 1985). Paul et al. (1983) reported an increase in unloaded shortening velocity on the addition of Ca^{2+} to thiophosphorylated strips. Although the present results (Fig. 4) showed a similar tendency the difference was not statistically significant, and could also be accounted for by the simultaneous small difference in force. Thus the results

from skinned smooth muscle preparations, while clearly showing an influence of Ca^{2+} on V_{max}, so far provide inconsistent evidence with respect to the concept that this influence is primarily exercised via Ca^{2+}-dependent effects on LC_{20} phosphorylation as proposed by Dillon et al. (1981). The effects at low-[MgATP], however, clearly do not fit into this picture. Here V_{max} is obviously limited by another mechanism, operating via an influence on the interaction of the activated cross-bridge with MgATP. According to current concepts of the biochemistry of the cross-bridge interaction, both in smooth and skeletal muscle, MgATP in solution will participate in the cross-bridge dissociation step (Marston 1983). The evidence from skinned skeletal muscle indicates that both V_{max} (Cooke and Bialek 1979; Ferenczi et al. 1984) and J_{ATP} (Takashi and Putnam 1979; Kushmerick and Krasner 1982) decrease at low-[MgATP] in a manner which is consistent with an elongation of the attached phase of the cross-bridge cycle. The present results from smooth muscle are consistent with this interpretation. However, the strong [MgATP] dependence of force in the smooth muscle (Fig. 2) indicates that the contractile system does not behave in a manner entirely analogous to that in skeletal muscle. Since we can now rule out the [MgATP] dependence of LC_{20} phosphorylation as a cause, the remaining explanation is that the effect is caused by differences in the kinetics of the cross-bridge interaction.

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