EFFECT OF INORGANIC PHOSPHATE ON THE CA²⁺ SENSITIVITY IN SKINNED *TAENIA COLI* SMOOTH MUSCLE FIBERS

Comparison of Tension, ATPase Activity, and Phosphorylation of the Regulatory Myosin Light Chains

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ABSTRACT Inorganic phosphate (P_i) decreases maximal tension in contracted skeletal and heart muscle fibers. We investigated the effects of 10 mM P_i on the force–calcium relationship in Triton X-100–skinned *Taenia coli* smooth muscle fibers. Isometric force measurements show that the calcium sensitivity of the force depends on the phosphate concentration. Furthermore 10 mM P_i relaxes the fibers more at intermediate than at high calcium ion concentrations: At pCa 4.5 tension decreases in the presence of 10 mM P_i by ~12% but it decreases 70% at pCa 6.17. Removal of phosphate partially reverses the relaxation. Simultaneous determination of actomyosin ATPase activity and force (Güth, K., and J. Junge, 1982, *Nature (Lond.)*, 300:775–776) shows that the ATPase activity does not correlate with the changes in force. In the presence of P_i, tension decreases more than the ATPase activity. The level of phosphorylation of the 20,000-D regulatory myosin light chain is not changed in the presence or absence of 10 mM P_i. The results are discussed in terms of slowly or noncycling myosin crossbridges formed at lower calcium concentrations, which contribute to the force development but not to the ATPase activity. These crossbridges are considered to be dissociated in the presence of phosphate.

INTRODUCTION

It is now generally accepted that force development results from a cyclic interaction of myosin crossbridges with the actin filament and it is assumed that the crossbridge cycle in smooth muscle is similar to that of skeletal muscle. Thus the crossbridge cycle in smooth muscle may be controlled by ATP, ADP, and inorganic phosphate (P_i) as is observed in skeletal muscle. Since it is assumed that during a complete crossbridge cycle one mole ATP per mole myosin is hydrolyzed, a measure for the cycle time of the actomyosin crossbridge interaction is given by the ATPase activity of the muscle. The reaction products of ATP cleavage by the ATPase reaction are P_i and ADP. P_i decreases isometric tension in skinned rabbit psoas fibers (Cooke and Pate, 1985; Godt et al., 1985; Kawai et al., 1985) but does not influence the maximum shortening velocity (Cooke and Pate, 1985). At maximal tension 10 mM P_i decreased the force in chicken gizzard fibers and shifted the Ca²⁺ sensitivity in soleus fibers (Kerrick and Hoar, 1985) and skinned ventricular trabeculae from rat (Kentish, 1985)

and hog (Herzig and Rüegg, 1977). In skinned smooth muscle preparations P_i accelerates relaxation after Ca²⁺ removal (Schneider et al., 1981).

During prolonged contraction P_i may accumulate within the muscle and therefore it could be an important factor in muscle fatigue or in ischemia (Jacobus et al., 1977; Godt et al., 1985). However, the quantity of P_i accumulation differs within a wide range. In ischemic heart, cytosolic P_i concentrations of 48 mM have been reported (Kübler and Katz, 1977). In resting skeletal muscle the concentration of P_i estimated by needle biopsy was 10 mmol/kg wet wt, while by ³¹P-nuclear magnetic resonance measurement, yields of 4 mM are obtained (Wilkie et al., 1984). In smooth muscle (hog carotid artery) the concentration of P_i was 3.8 μmol/g blot wt in the relaxed state and 5 μmol/g blot wt during contraction (Krisanda and Paul, 1983).

Smooth muscle contraction is initiated by an increase of intracellular Ca²⁺, which activates the Ca²⁺- and calmodulin-dependent myosin light chain kinase (MLCK). Upon phosphorylation of the regulatory 20,000-D myosin light chains by the MLCK, inhibition of actomyosin interaction is relieved. The importance of myosin light chain phosphorylation for smooth muscle contraction was demonstrated

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using skinned fiber preparations: In the absence of Ca^{2+} addition of a Ca^{2+}- and calmodulin-independent form of the MLCK led to contraction (Walsh et al., 1982; Gagelmann et al., 1984a) and enabled actomyosin interaction (Mrwa et al., 1985). After Ca^{2+} is withdrawn from contracted skinned smooth muscle preparations, ATPase activity rapidly drops to resting level while force slowly decays. Addition of P_i speeds up relaxation (Güth and Junge, 1982; Güth et al., 1984). The observation that after Ca^{2+} removal tension is maintained for a considerable period of time without ATPase activity led to the suggestion that the remaining tension is due to noncycling myosin crossbridges (Güth and Junge, 1982).

Dephosphorylation of the regulatory myosin light chains of attached crossbridges was considered to arrest the crossbridge cycle, forming attached crossbridges, termed latch-bridges (Dillon et al., 1981; Akosy et al., 1982). Later, Chatterjee and Murphy (1983) presented experiments indicating that a second Ca^{2+}-dependent regulatory mechanism seems to be involved in formation of latch-bridges and that this system has a higher sensitivity to Ca^{2+} than does MLCK.

Therefore it was of interest to study the conditions necessary for initiation of noncycling but force-generating crossbridges in smooth muscle. Because of the peculiar effects of P_i during muscular contraction we wanted to find out whether P_i shifts the calcium sensitivity of force and whether it influences ATPase activity in smooth muscle. Therefore, in this work, force and ATPase activity were studied simultaneously in skinned smooth muscle preparations. Our data demonstrate that noncycling crossbridges may be formed by lowering the Ca^{2+} concentration in a contracted smooth muscle. Further, it is reported that P_i influences the pCa–force–relationship but not actin-activated ATPase activity. The results are discussed in terms of a model that assumes formation of latch-bridges when the Ca^{2+} concentration decreases from higher to lower levels. Since formation of latch-bridges contributes to the force but not to the ATPase activity while P_i decreases force without appropriate change of ATPase activity formation of noncycling crossbridges may depend on both the Ca^{2+} and phosphate concentrations.

MATERIALS AND METHODS

Skinned Fiber Preparation

Smooth muscles from guinea pig *Taenia coli* were chemically skinned with Triton X-100 as described earlier by Sparrow et al. (1981).

Preparation of Solutions

The solutions were calculated with a computer program as reported by Fabiato and Fabiato (1979) and also the stability constants as given by the authors were used. In all solutions the ionic strength was adjusted to 100 mM by KCl. Relaxing solution (pCa 8): 20 mM imidazole, 7.5 mM Mg-ATP, 1 mM free Mg^{2+}, 4 mM EGTA, 1 mM phosphoenol pyruvate (PEP), 1 mM NaN_3. Contracting solution (pCa 4.5): 20 mM imidazole, 7.5 mM Mg-ATP, 1 mM free Mg^{2+}, 4 mM Ca-EGTA, 1 mM PEP, 1

mM NaN_3. The Ca^{2+}-jump technique was applied when the Ca^{2+} concentration was changed from high to low levels. This technique allows to change the Ca^{2+} concentration rapidly in small bundles of chemically skinned muscle fibers while all other concentrations are held constant (Moisescu, 1976). In the Ca^{2+}-jump technique the fibers were preincubated (before change from pCa 4.5 to higher pCa) with the corresponding preincubation solution (Ca^{2+}-jump solution), which had the same composition as the contracting solution except that the concentration of EGTA was lowered from 4 mM to 0.2 mM EGTA and 3.8 mM 1,6-diaminohexane-N,N,N',N'-tetraacetic acid (HDTA) was added, which is unable to complex Ca^{2+}. Solutions containing intermediate Ca^{2+} concentrations were obtained by mixing relaxing and contracting solutions. To all solutions 0.1 μM calmodulin (prepared according to Gopalakrishna and Anderson, 1982) and 100 U/ml pyruvate kinase (EC 2.7.1.40, Boehringer Mannheim GmbH, Mannheim, FRG) was added. The pH of the solutions was adjusted to pH 6.7 and experiments were carried out at 22°C. The concentration of P_i was 10 mM. For the ATPase measurements the solutions additionally contained 0.6 mM NADH, 1 mM dithioerythritol (DTE), 140 U/ml lactate dehydrogenase (EC 1.1.1.27, Boehringer Mannheim GmbH), and 0.2 mM P1,P5-(adenosine-5'-) pentaphosphate (myokinase inhibitor).

Mechanical Measurements

Smooth muscle fiber bundles ($\sim$350-μm diam) were mounted horizontally (6-mm long) between a movable glass rod and a Statham UC 2 force transducer (H. Sachs Elektronik, March-Hugsletten, FRG). The fiber bundles were bathed in the appropriate solutions in 1-ml lucite chambers
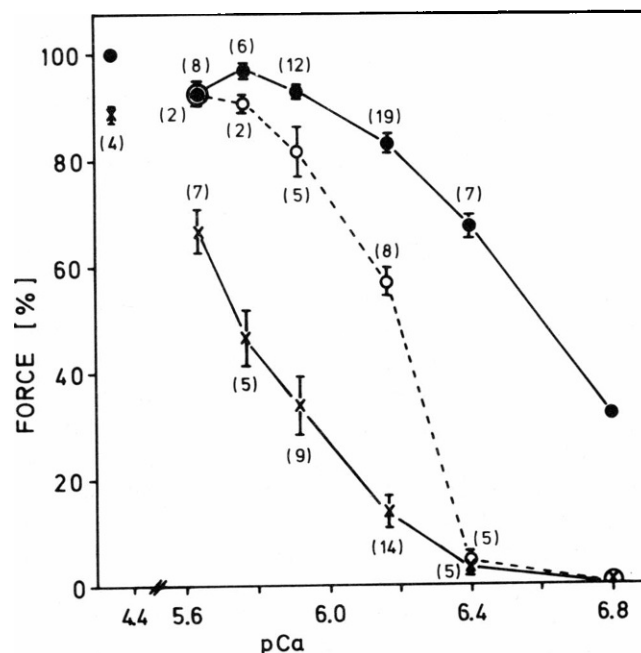

FIGURE 1   Effect of P_i on the tension. After contraction at pCa 4.5 the fibers were changed into solutions containing various Ca^{2+} concentrations and the force level was determined after 10-min incubation (*solid circles*). During this time almost all fibers reached a plateau in tension. By keeping the calcium concentration and ionic strength constant the effect of 10 mM P_i was studied after 10-min incubation in the P_i-containing solution (*crosses*). The effect of P_i was reversed after its removal (*open circles*). The tension redevelopment was not complete within the 10-min incubation period. Values are mean values ($\pm$SE) obtained from different fibers preparations as indicated by the numbers in the figure.

and the solutions were changed by moving the fiber from one solution chamber to another.

Experimental Protocol for Force and Regulatory Myosin Light Chain (LC) Phosphorylation Measurements

The experimental protocol used to study the effects of P_i was as follows (compare also Fig. 2, *top*): The fibers were incubated under isometric conditions in relaxing solution (step 1) and a contraction was initiated by raising the Ca^{2+} concentration from pCa 8 to pCa 4.5 (step 2). After 20–30 min when the fibers were completely contracted, they were incubated for 10 min in solutions containing various free Ca^{2+} concentrations (step 3). In step 4 the incubation solution contained 10 mM P_i at the

same ionic strength and without change of Ca^{2+} concentration. Incubation of the fibers was for 10 min. To demonstrate reversibility of the phosphate effect, P_i was withdrawn again in step 5. Then the fibers were contracted again at pCa 4.5 (step 6) and subsequently relaxed (step 7) at pCa 8. A similar experimental protocol was described earlier for determination of cAMP-induced relaxation in smooth muscle (Meisheri and Rüegg, 1983). It was pointed out by the authors that this protocol allows much more rapid attainment of constant force levels (plateau tension) at submaximal Ca^{2+} concentrations. The protocol also avoids repeated contraction–relaxation cycles, which decrease the contractility of the skinned fibers (Endo et al., 1977; Rüegg and Paul, 1982; Sparrow et al., 1984). In the absence of P_i in almost all fibers the plateau level of tension was nearly achieved after 10 min. In the presence of 10 mM P_i constant force levels were not achieved within 10 min, independent of the Ca^{2+} concentration. Nevertheless, to prevent a degradation of the tension
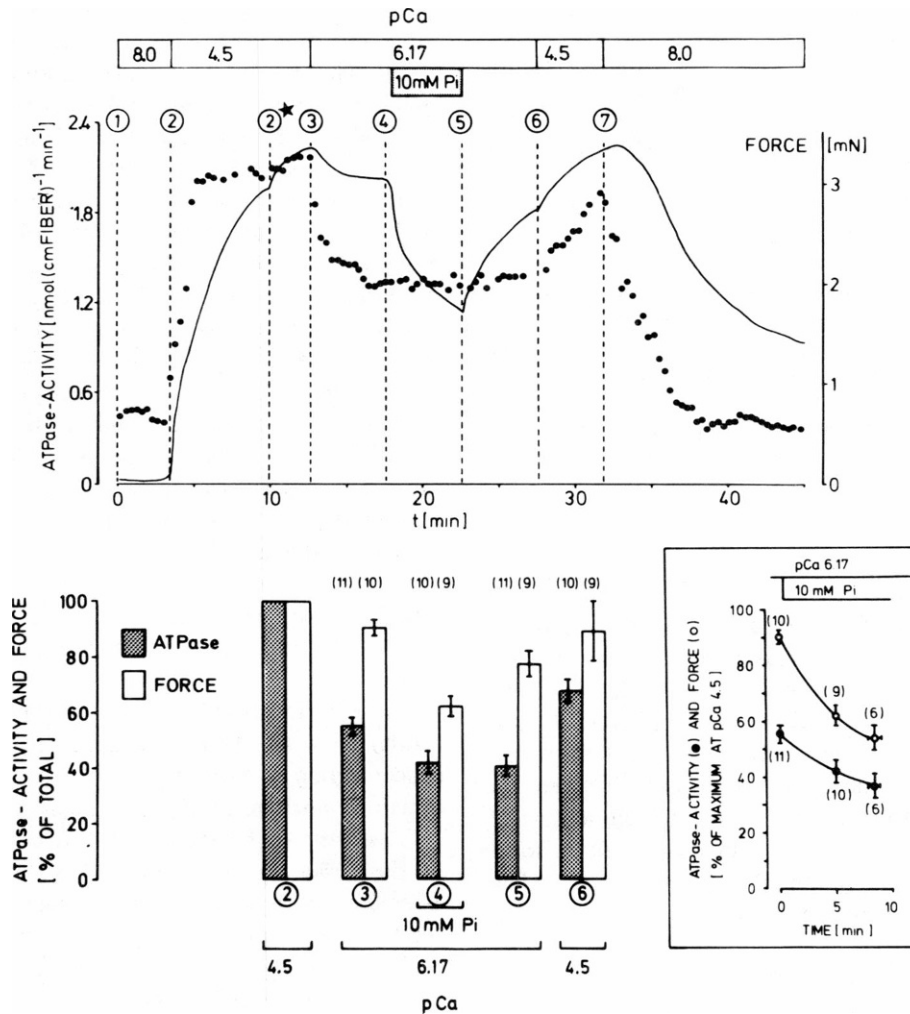

FIGURE 2 ATPase activity at pCa 6.17 with and without 10 mM P_i. (*Top*) A recording of the tension (*curve*) and the ATPase activity (*solid circles*) measured simultaneously in a skinned *Taenia coli* fiber bundle. The ATPase activity was determined every 20 s. The experimental procedure was as described in the Material and Methods section except that the incubation times were reduced (5 min). The fibers were mounted in the absence of Ca^{2+} at pCa 8.0 (step 1) and contracted with pCa 4.5 (step 2). To change the Ca^{2+} concentration rapidly in step 3 the Ca^{2+}-jump technique was used (step 2*). After changing to pCa 6.17 (step 3) P_i (10 mM) was added (step 4) and removed (step 5). For control, fibers were contracted again at pCa 4.5 (step 6) and relaxed by removal of Ca^{2+} (step 7). (*Bottom*) Summary of different experiments (the number of experiments is given on top of the bars) showing the mean values (±SE) of the ATPase activities (*stippled*) determined during the last minute at the end of the corresponding incubation steps (steps are indicated by the numbers in the circles on the bottom of the bars) just before the solutions were changed. (Basal ATPase activity at pCa 8 was subtracted.) The according forces were determined at the end of the appropriate steps (*open bars*). The *inset* at bottom right shows the activities of ATPase (*solid circles*) and force (*open circles*) determined simultaneously at pCa 6.17 without (zero time) and with 10 mM P_i after 5 and 8.5 ± 0.5 min. (Values are mean values ±SE from different fibers.)

because of prolonged contractions as reported by Sparrow et al. (1984) in the presence of 10 mM P_i, the incubation time was terminated at 10 min. Correspondingly the analyses of LC phosphorylation were also performed after 10 min incubation time.

Fiber preparations showing no steady-state force after lowering Ca^{2+} from pCa 4.5 to 6.17 in step 3 (in the absence of P_i) showed little LC phosphorylation. These fibers were discarded. Some of the fibers being relaxed by P_i did not contract after removal of P_i. LC phosphorylation in these fibers revealed no or little LC phosphorylation. Therefore, these fiber preparations were also discarded.

ATPase Activity

The ATPase activity in the skinned fibers (200–300-μm diam) was determined every 20 s in a narrow chamber by perfusion with the solutions as described by Güth and Junge (1982) and Güth and Wojcie-chowski (1986). Because of the high expense of enzymes and substrates used for the NADH-coupled optical assay method of the ATPase activity determination, the incubation times were reduced. The experimental protocol was similar to the one described above except that the Ca^{2+}-jump technique (step 2*) was used for the change of the Ca^{2+} from high (pCa 4.5) to intermediate concentrations at step 3. Therefore the fibers were incubated at low calcium-EGTA concentration (step 2*) before the Ca^{2+} concentration was changed.

Determination of LC Phosphorylation

The phosphorylation of the regulatory LC was determined by two-dimensional gel electrophoresis according to O'Farrell (1975) with modifications as described by Gagelmann et al. (1984b). Details for termination of the chemical reactions within the fibers and homogenization were as described Gagelmann and Güth (1985).

A preliminary report of this study has been presented as an abstract (Gagelmann et al., 1986).

RESULTS

Effect of P_i on the Ca^{2+}–Force Relationship

Fig. 1 shows the force generated in chemically skinned fibers of *Taenia coli* smooth muscle in the presence and absence of 10 mM P_i. The experimental procedure was as given in detail in the Materials and Methods section (see also Fig. 2). It can be seen from Fig. 1 that the Ca^{2+} sensitivity of the force developed is largely dependent on whether or not phosphate is added to the solutions. The force before addition of P_i to the incubation solution (*solid circles*) is reduced when 10 mM P_i is added (*crosses*). The removal of P_i partly reversed the relaxation (*open circles*).

Effect of P_i on the ATPase Activity

ATPase activity and force were measured simultaneously in the same fiber preparation as described earlier (Güth and Junge, 1982). It is evident from the results demonstrated in Fig. 1 that phosphate relaxes the fibers mostly at intermediate Ca^{2+} concentrations. Therefore the phosphate effect on the ATPase activity was investigated at pCa 6.17. A recording of force and ATPase activity is given in Fig. 2 (*top*). Fig. 2, *bottom* summarizes different experiments showing the mean ($\pm$SE) of the ATPase activities (*stippled bars*) determined at the end of the corresponding incubation periods just before the solution

was changed. The corresponding force levels are given by the open bars. After Ca^{2+} activation (step 2) the force and the ATPase activity increased. As reported earlier the ATPase activity increased faster than the force (Gagel-mann and Güth, 1985). To apply the Ca^{2+}-jump technique the fibers were incubated at low calcium-EGTA concentration (step 2*) as described in Materials and Methods. Then in step 3 the Ca^{2+} concentration was changed rapidly from pCa 4.5 to 6.17. The ATPase activity decreased (within 5 min) to 55.0 $\pm$ 3.4% of the maximal ATPase activity ($n = 11$). The force also decreased (90.4 $\pm$ 2.8%, $n = 10$) but by a smaller amount than the ATPase activity. When, in step 4, P_i (10 mM) was added, the force decayed again to 62.0 $\pm$ 3.8% ($n = 9$). (Compared with the force values demonstrated in Fig. 1 the drop in the force was less here.) The fall in force after addition of P_i was not associated with a corresponding change in the ATPase activity. Fig. 2 shows that the ATPase activity is rather constant after addition of 10 mM P_i in step 4. On the average, in 10 fibers a slight decay of the activity was observed. This can also be seen from Fig. 2, *bottom right*: After a 5 min incubation in the presence of 10 mM P_i the ATPase activity was 42.0 $\pm$ 4.1% ($n = 10$), after 8.5 $\pm$ 0.5 min it was 36.7 $\pm$ 4.4% ($n = 6$). Also the force does not reach a plateau within 5 min (see Fig. 2, *top*, and Fig. 2, *bottom right*). Therefore the steady-state tension corresponding to 10 mM P_i could not be determined. The force at the end of the incubation period in the presence of P_i (at the end of step 4) was 62.0 $\pm$ 3.8% ($n = 9$) of the maximal tension at pCa 4.5, whereas it was 54.0 $\pm$ 4.5% ($n = 6$) after 8.5 $\pm$ 0.5 min. After removal of the P_i force increased again to 77.2 $\pm$ 4.6% ($n = 9$). This increase in force was not associated with an increase of the ATPase activity, which was 40.6 $\pm$ 3.9% ($n = 11$) at the end of the incubation period.

The experiment was repeated at a higher Ca^{2+} concentration ($n = 4$). After contraction at pCa 4.5 the Ca^{2+} concentration was lowered to pCa 5.92. While the force remained unchanged (99.9 $\pm$ 2.1%, see also Fig. 1) the ATPase dropped to 70.5 $\pm$ 4.8%). After addition of 10 mM P_i the force decay obtained (75.3 $\pm$ 1.2%) was not associated with a concomittant change in the ATPase (60.5 $\pm$ 8.9%) during the 5-min incubation. After removal of P_i the ATPase activity determined after 5-min incubation) was 63.6 $\pm$ 5.8% while the force again reached the maximum (99.9 $\pm$ 2.1%).

Phosphorylation of the Regulatory LC

We investigated LC phosphorylation with and without P_i (10 mM) at pCa 6.17 (Table I). During maximal contraction at pCa 4.5 the total LC phosphorylation increased from 13.9 $\pm$ 5.7% ($n = 10$, at pCa 8) to 62.5 $\pm$ 6.1% ($n = 22$). The change from pCa 4.5 to pCa 6.17 was associated with dephosphorylation of the regulatory myosin light chains to 24.6 $\pm$ 3.1% (eight fibers, determination after 10 min incubation). After addition of 10 mM P_i for 10 min the

TABLE I

COMPARISON OF THE LC-PHOSPHORYLATION IN THE RELAXED (pCa 8), CONTRACTED STATE (pCa 4.5), AND AT INTERMEDIATE CA²⁺-ION CONCENTRATION (pCa 6.17) AND THE EFFECT OF 10 MM P_i at pCa 6.17

Treatment of skinned fibers	LC-phosphorylation	No. of experiments
	% of total, ±SE	
A Relaxed state (pCa 8)	13.9 ± 2.9	10
B Contraction at pCa 4.5 (20–30 min)	62.5 ± 1.3	22
C Change to pCa 6.17 (10 min) after contraction at pCa 4.5	24.6 ± 3.1	8
D Incubation with 10 mM P_i at pCa 6.17 (10 min)	21.0 ± 1.9	10
E Removal of P_i (pCa 6.17, 10 min)	22.0 ± 1.9	9

level of phosphorylation decreased slightly but not significantly to 21.0 ± 6.1% ($n = 10$) and when P_i was removed the LC phosphorylation was found to be 22.0 ± 5.8% ($n = 9$).

DISCUSSION

It is shown in Fig. 1 that the Ca^{2+} sensitivity of the force in *Taenia coli* smooth muscle fibers depends on the phosphate concentration within the incubation solution. In the presence of 10 mM P_i the fibers are less sensitive to Ca^{2+}. Removal of P_i reverses this shift in the pCa–force relationship. The force is also lowered at saturating Ca^{2+} concentrations, but the effect of P_i is more pronounced at intermediate Ca^{2+} levels (pCa 5.8 to 6.4). The observed decrease in calcium sensitivity of force generation in the presence of P_i has also been reported for skinned soleus muscle and chicken gizzard (Kerrick and Hoar, 1985), rabbit psoas (Kawai et al., 1985; Cooke and Pate, 1985), and heart trabeculae muscle (Kentish, 1985; Herzig and Rüegg, 1977). It is suggested that the shift in the Ca^{2+} sensitivity of the force is based on an altered Ca^{2+} affinity of the regulatory proteins, which may be caused by phosphate-mediated changes in the actomyosin interaction cycle (Kerrick and Hoar, 1985). However, if the Ca^{2+} sensitivity of the regulatory protein is different in the presence or absence of phosphate, one would expect that the Ca^{2+} sensitivity of the ATPase activity of the muscle would be changed in parallel.

In contrast to the force, the ATPase activity is not significantly different in the presence or absence of P_i either at high (data not shown) or at intermediate Ca^{2+} concentrations. It may be concluded that the P_i-mediated shift of the pCa–force relationship does not exist for the ATPase activity pCa–relationship: A possible conclusion is that in smooth muscle, force and ATPase activity are regulated differently.

When the Ca^{2+} concentration during contraction is lowered from pCa 4.5 to pCa 6.17 the force after 5-min incubation is still high (90.4 ± 2.7%, $n = 10$), while a considerable decrease of the ATPase activity to 55.0 ± 3.4 ($n = 11$) is observed. (The force level determined simultaneously with the ATPase is a little higher than measured under isometric conditions in Fig. 1 [83.0 ± 1.7%, $n = 19$]. This may be due to the comparatively short incubation period at pCa 4.5 preceding the change to pCa 6.17 in the ATPase experiments, which was not sufficient to reach a plateau of force. In contrast the force was allowed to reach a plateau in the experiments in Fig. 1.) Fig. 2 shows that at pCa 6.17 the addition of 10 mM P_i (5 min) decreases the ATPase activity only a little further (Fig. 2, *bottom* and *inset*), but it causes a considerable drop in tension. We reported earlier (Güth and Junge, 1982) that after withdrawal of Ca^{2+} from the fiber non- or slowly cycling (but force-generating) crossbridges are formed with the actin filament. We showed also that phosphate increased the dissociation rate of these crossbridges (see also Schneider et al., 1981; Güth et al., 1984). Therefore it is tempting to conclude that such noncycling but force-generating crossbridges may also be formed at intermediate Ca^{2+} concentrations (Chatterjee and Murphy, 1983). The number of these crossbridges may depend on both the Ca^{2+} concentration and the phosphate concentration, i.e., at intermediate Ca^{2+} concentrations the tendency to form noncycling but force-generating crossbridges is high in the absence of P_i. But the noncycling crossbridges may be dissociated in the presence of P_i. This model is consistent with our results: At intermediate Ca^{2+} concentrations the number of cycling crossbridges is lowered and thus the ATPase activity is also lower. Since, however, the tendency to noncycling but force-generating crossbridges is high, the force decreases less than the ATPase activity. The addition of P_i reduces the number of noncycling but force-generating crossbridges, affecting the force but only to a small degree the ATPase activity. Consistent with this interpretation the force increases again, when at intermediate Ca^{2+} concentrations the P_i is withdrawn from the incubation solution. In contrast the ATPase activity again is not much affected.

When the noncycling but force-generating crossbridges are dissociated by phosphate, one might expect that these crossbridges might participate in additional interaction cycles, thus causing an increased ATPase activity in the presence of P_i. However, it is unknown whether these crossbridges are conditioned to cycle again after they are dissociated by P_i. Furthermore, it is not clear how much force the noncycling crossbridges may generate per crossbridge. Thus in the absence of P_i a small number of noncycling crossbridges may generate much force. In this case the dissociation of these crossbridges would cause only a small increase in ATPase, even if they participate in the ATPase cycle. Because of these reasons more data must be obtained about the nature of the noncycling crossbridges before further conclusions can be drawn.

Phosphorylation of the regulatory myosin light chains, which was proposed to be correlated with the change from cycling to noncycling force-generating crossbridges in living smooth muscle (Dillon et al., 1981; Aksoy et al., 1982), seems not to be involved in the regulation by P_i described in the present study, since no change in the level of light chain phosphorylation was observed in the presence or absence of P_i. Therefore the decreased Ca^{2+} sensitivity of force generation in the presence of P_i is not related to inhibition of the myosin light chain phosphorylation or stimulation of dephosphorylation.

In the following discussion, some problems concerning the reliability of our data are considered. A discrepancy between the force levels at pCa 6.17 determined under isometric conditions (Fig. 1) and in the ATPase experiments (Fig. 2) is observed: After 5-min incubation with P_i the force was $25.7 \pm 2.7\%$ ($n = 12$) under isometric conditions (data not shown), and then decreased further to $13.9 \pm 3.0\%$ ($n = 14$) within the following 5 min. In contrast, the force obtained simultaneously with the ATPase decreased only to $62.0 \pm 3.8\%$ ($n = 9$). The reason for the smaller tension decay in the ATPase experiments is not clear, but it should be emphasized that it is nevertheless evident that the ATPase activity is only slightly affected when the Ca^{2+} concentration is changed, while the force drops significantly. Prolongation of the incubation time (8.5 ± 0.5 min) reveals a further (but small) decay of the ATPase and a concommittant decay of the tension. From the experiments it is difficult to conclude whether this further change of ATPase activity and force is significant. But it should be noted that a slow transient decay of the ATPase during the experiment has to be considered: During continuous (Gagelmann and Güth, 1985) or repeated contractions (Mrwa et al., 1985), the maximal ATPase activity (pCa 4.5) often transiently decays. This can also be seen from Fig. 2: The ATPase in the second contraction after 5 min was found to be only $67.5 \pm 4.0\%$ ($n = 10$) of the activity determined during the initial contraction.

There might be concern that the increase of the total ATPase activity with increasing Ca^{2+} consists of (a) a Ca^{2+}-activated ATPase that is not related to the actomyosin interaction (nonspecific ATPase) and (b) the Ca^{2+}- and actin-activated myosin ATPase. However, we showed recently in collaboration with other laboratories (Mrwa et al., 1985) that in Triton X-100–skinned *Taenia coli* smooth muscle only the actomyosin ATPase is activated during contraction in the presence of Ca^{2+}: Incubation with a Ca^{2+}- and calmodulin-independent MLCK elicited in the absence of Ca^{2+} an almost complete contraction and maximum ATPase activity. Addition of Ca^{2+} (pCa 4.5) resulted in only a slight (10%) increase of both the maximal developed ATPase activity and force. Therefore the fibers were probably not completely activated by the Ca^{2+}- and calmodulin-insensitive MLCK, and consequently the small Ca^{2+}-dependent 10% increase in force and ATPase activity is probably caused by an activation of

the intrinsic Ca^{2+}-dependent MLCK. Because of the apparent lack of a Ca^{2+}-regulated component of the ATPase activity in the presence of Ca^{2+}-insensitive MLCK, it can be concluded that the contribution of the nonspecific ATPase can be neglected in our experiments.

It might be suggested that P_i could act on a non-actomyosin ATPase activity, which is not Ca^{2+}-sensitive. The stimultion of this nonspecific ATPase by P_i could then mask a decrease of the actomyosin ATPase in our experiments. However, we showed earlier that (in the absence of Ca^{2+}) no difference in the ATPase activity was observed in the presence or absence of P_i (Güth and Junge, 1982; Güth et al., 1984).

In summary, P_i lowers the calcium sensitivity of force development in skinned smooth muscle fibers. The phosphate-mediated shift in the calcium sensitivity of force is not associated with a corresponding change in the actin-activated ATPase activity. Therefore, in smooth muscle ATPase activity and force may be regulated differently. The results are consistent with a model that assumes formation of attached crossbridges at submaximal calcium concentrations which contribute to the force but not to the ATPase activity. P_i reduces the number of noncycling crossbridges (latch-bridges). Thus, at submaximal Ca^{2+} concentrations and in the presence of P_i the force drops without a corresponding change of the ATPase activity.

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