Nucleotide-Dependent Contractile Properties of Ca^{2+}-Activated Fast and Slow Skeletal Muscle Fibers

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ABSTRACT The relation between single skinned skeletal fiber contractile mechanics and the myosin mechanoenzyme was examined by perturbing the actomyosin interaction with the ATP analog CTP in fibers from both rabbit psoas and rat soleus. Tension, instantaneous stiffness, and the rate of tension redevelopment (k_{tr}), under software-based sarcomere length control, were examined at 15°C for a range of Ca^{2+} concentrations in both fiber types. CTP produced 94% of the maximum ATP-generated tension in psoas fibers and 77% in soleus fibers. In psoas, CTP also increased stiffness to 106% of the ATP stiffness, whereas in soleus stiffness decreased to 92%. Thus, part of the greater difference between maximum ATP- and CTP-generated tension in soleus fibers appears to be due to a decrease in strongly bound cross-bridge number. Interestingly, although the nucleotide exchange produced substantial increases in the steepness (n_H) of the tension- and stiffness-pCa relationships in soleus fibers, only minor changes were seen in psoas fibers. At maximum Ca^{2+} and nominal P_i levels, k_{tr} in psoas fibers increased from 11.7 s^{-1} with ATP to 16.6 s^{-1} with CTP and in soleus fibers from 4.9 to 8.4 s^{-1}. Increased P_i levels decreased the maximum Ca^{2+}-activated tension in both fiber types and increased the k_{tr} of psoas fibers, but the k_{tr} of soleus fibers was not significantly altered. Thus, although the nucleotide exchange generally produced similar changes in the mechanics, there were significant muscle lineage differences in the tension- and stiffness-pCa relations and in the effects of P_i on k_{tr}, such that differences in contractile mechanics were lessened in the presence of CTP.

INTRODUCTION

Comparative studies of fast and slow skeletal muscle indicate a wide variation in the maximum rates of tension development (~8-fold; Metzger and Moss, 1990) and shortening velocity (Barany, 1967). In skinned fibers these differences in contractility are expected to be directly related to the differential protein expression patterns observed for the contractile machinery. More specifically, differences in the rates of contraction in muscle fibers are expected to be closely coupled to differences in the cross-bridge cycling rates of different myosin isoforms (Reiser et al., 1985; Siemankowski et al., 1985; Metzger and Moss, 1990).

Cross-bridge cycling, and hence contraction of skeletal muscle, is powered by the splitting of ATP. The skinned fiber preparation has been widely used to elucidate the coupling between the actomyosin ATPase and the mechanics of muscle contraction by allowing perturbation of the ATPase with simultaneous mechanical measurements. Studies both in solution and in skinned fibers have led to Scheme 1 for the actomyosin ATPase cycle (for reviews see Goldman, 1987; Homsher and Millar, 1990; Taylor, 1992). This scheme consists of weak binding (AM·ATP and AM·ADP·P_i) states that bear little or no tension and are in rapid equilibrium with unbound states, followed by a transition to strong, tension-bearing states (AM′·ADP through AM). The coupling of the actomyosin ATPase to tension production has been well studied in fast skeletal muscle. However, there have been few studies of the coupling mechanism in slow skeletal muscle fibers. We hypothesize that differences in the mechanical properties of different muscle fiber types correspond to differences, either kinetic or structural, in specific states of the cross-bridge cycle.

There are significant differences in the observed cooperativity of Ca^{2+} activation between different fiber types as well, with the cooperativity being greater in fast fibers (Kerrick et al., 1976; Metzger and Moss, 1987). It has been proposed that cross-bridges may play a key role in activating the thin filament, possibly by promoting the movement of tropomyosin from an "off" to an "on" conformation (Hill et al., 1983; McKillop and Geeves, 1993). It has also been demonstrated that there are reciprocal interactions between the cross-bridges and the thin filament that may lead to an increase in thin filament activation (Gordon and Ridgway, 1987; Güth and Potter, 1987). Furthermore, these reciprocal interactions appear to be dependent on specific cross-bridge states. In this way differences in the cross-bridge cycle may underlie part of the difference in the cooperativity of Ca^{2+} activation. It is therefore of interest to investigate whether perturbations of the cross-bridge cycle lead to perturbations in the cooperativity of Ca^{2+} activation.

The purpose of this study is to examine changes in the mechanics of fast and slow muscle fibers after perturbation of the cross-bridge cycle. This perturbation is accomplished through the use of the ATP analog CTP to alter the mecha-

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$$\begin{array}{ccccccccc} AM+ATP & \leftrightarrow & AM.ATP & \leftrightarrow & AM.ADP.P_i & \leftrightarrow & AM'.ADP & \leftrightarrow & AM.ADP & \leftrightarrow & AM \\ & & \updownarrow & & \updownarrow & & & & & & \\ & & M.ATP & \leftrightarrow & M.ADP.P_i & & & & & & \end{array}$$

SCHEME 1

noenzyme. Myosin is capable of producing active tension with a number of both nucleotide and nonnucleotide analogs of ATP (Pate et al., 1991, 1993). In skinned fast skeletal muscle fibers CTP produces amounts of active tension and stiffness similar to those produced by ATP, but significantly reduces the maximum rate of unloaded shortening (Pate et al., 1993). However, in solution the actomyosin CTP hydrolysis rate is 50% more rapid than the ATP hydrolysis rate (White et al., 1993). Thus, the use of CTP causes significant changes in the kinetics of the cross-bridge cycle. In this study we have extended the investigation of the mechanical responses to a change in nucleotide to include both fast and slow fiber types for the first time. In addition, we examined the relationship between the contractile mechanics and the level of Ca^{2+} activation. In this way we reveal important differences in the mechanoenzyme of fast and slow skeletal muscle that have direct consequences for both the kinetics of contraction and the Ca^{2+}-activation properties of skeletal muscle fibers.

MATERIALS AND METHODS

Skinned fiber preparations

Slow-twitch fibers were obtained from the soleus muscle of adult female Sprague-Dawley rats and fast-twitch fibers from the psoas muscle of adult male New Zealand rabbits. Small bundles of fibers were isolated from the muscle under cold relaxing solution and tied to glass capillary tubes. These bundles were then placed in relaxing solution containing 50% glycerol at 4°C overnight. The bundles were then stored at −20°C until use. Fibers were used within a month of dissection.

Individual fibers were carefully pulled from the bundle and mounted between a force transducer (model 400A; Cambridge Technology, Cambridge, MA) and a galvanometer (Cambridge Technology) as described previously (Metzger et al., 1989). The sarcomere length was adjusted to 2.50–2.55 μm by adjusting the overall fiber length with a mechanical translator. Cross-sectional area was determined from measurements of the width and depth of the fiber by assuming an elliptical cross section. A 10-mW HeNe laser was directed through the fiber from beneath the chamber, and the first-order diffraction line was detected by a lateral-effect photodiode (model LSC-5D; UDT Sensors, Hawthorne, CA). The output of the photodiode was used to provide feedback control of the sarcomere length via the galvanometer. This feedback control was accomplished through a custom-designed computer program that also collected and stored simultaneous records of the tension, galvanometer deviation, and diffraction line position for later analysis.

Closed-loop computer tension feedback control

A computer software system was created that measured sarcomere spacing by monitoring the diffraction of laser light through the fiber. In addition, the software simultaneously monitored the tension and the galvanometer position. The system was calibrated by adjusting the length of the cell by applying a ramp voltage to the galvanometer and calculating the change in sarcomere length based upon the length of the preparation, the sarcomere length, and the number of sarcomeres along the fiber. In open loop operation, the software was capable of monitoring the sarcomere spacing and strain on the fiber at 16,000 Hz. The software system was also capable of performing closed-loop feedback control of either sarcomere spacing or tension. Feedback control was achieved through the use of a re-entrant software routine that compared the control set point of sarcomere length or strain on the fiber with the current measured value, and adjusting the galvanometer in an arithmetic fashion, at the rate of 2000 Hz. The results of closed-loop control were displayed graphically for visual confirmation of control. The software then normalized and saved the data for off-line analysis. To prevent damage to the fiber, the maximum distance and rate of galvanometer movement were limited by the software.

Instantaneous stiffness

Instantaneous stiffness was determined by applying a small (~0.1% of total fiber length) 1-kHz sinusoidal length change to the preparation. The amplitudes of ≥ 20 peaks in the resulting tension and length traces were then averaged. The instantaneous stiffness was defined as the average change in tension divided by the average change in length for each activation level. The value for stiffness obtained in relaxing solution (pCa 9.0) was subtracted from the value in activating solutions to obtain the active stiffness. Increases in the amplitude of the instantaneous active stiffness were assumed to be proportional to increases in the number of strongly bound cross-bridges (Huxley and Simmons, 1972).

Solutions

ATP-containing solutions were prepared using the program of Fabiato (1988) to calculate final concentrations of metals, ligands, and metal-ligand complexes. These solutions contained 7 mM EGTA, 14.5 mM creatine phosphate, 20 mM imidazole, and 4 mM MgATP, pMg 3.0, at 15°C. The pCa was set in a range from 4.5 (maximum activation) to 9.0 (relaxing). Inorganic phosphate (0 to 30 mM) was added as KH_2PO_4. The concentration of phosphate within the fiber due to P_i contamination in the solutions and due to the hydrolysis of nucleotide triphosphate by the fiber was estimated to be ~0.7 mM (Pate and Cooke, 1989). KCl was added to bring the total ionic strength to 180 mM, and the pH was adjusted to 7.0 with KOH. CTP-containing solutions were identical, except that ATP was replaced with CTP. It was assumed that the H^+ and metal binding constants of CTP were identical to those of ATP (Pate et al., 1993).

Rate of tension redevelopment

The rate of tension redevelopment (k_{tr}) (Brenner and Eisenberg, 1986) was measured by activating the fiber and introducing a release (10–15% of total fiber length) after the tension reached a steady state, allowing the fiber to shorten for 10 ms, followed by a rapid restretch to mechanically dissociate the myosin cross-bridges from actin (Metzger et al., 1989). The rate constant of the resulting tension redevelopment is defined as k_{tr}. Unless otherwise noted, feedback control of the first-order laser diffraction line was used during the tension redevelopment to maintain the sarcomere length of the preparation at a constant value, thus simplifying the interpretation of the results.

Experimental protocol

The general experimental protocol is shown in Fig. 1. The first, last, and every third or fourth activation were at maximum Ca^{2+} (pCa 4.5) to provide a control. Changes in tension between these maximum activations were assumed to be linear and decreased by less than 10%. Submaximum activations were given in no particular order. Likewise, the order in which the nucleotides were used was varied. The effects observed under all experimental conditions were fully reversible. To prevent contamination when exchanging nucleotides, the fiber and the chambers were washed twice with relaxing solution containing the new nucleotide. Each experiment was performed with alternating ATP and CTP solutions in the same fiber, thus allowing each fiber to serve as its own control and minimizing the effects of interfiber variation.

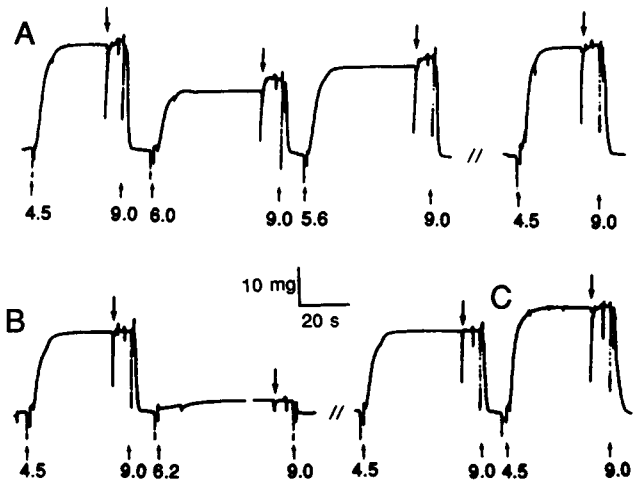

FIGURE 1 Slow time base recording of tension development of a single skinned rat soleus fiber in response to activating solutions of varying pCa (only a small number of representative traces are shown). Changes in pCa are indicated below the trace. The fiber was first activated at pCa 4.5 as a control (A); at the peak of tension development the fiber was rapidly slackened and restretched (*arrows*) and then relaxed by returning to relaxing solution (pCa 9.0). The fiber was then subsequently activated at lower pCa and relaxed as shown. After several submaximum pCa activations, the fiber was again activated at pCa 4.5 to check for deterioration in the tension production. At B the fiber was washed twice with relaxing solution containing CTP, and the protocol was repeated. Reversibility is demonstrated at C by washing twice with relaxing solution containing ATP followed by a pCa 4.5 activation.

Curve fitting and statistics

Curve fitting was performed using the computer program Igor (WaveMetrics, Lake Oswego, OR). The k_{tr} was derived by fitting the tension trace to an exponential equation of the form $P = P_{init} + A \exp(-k_{tr}t)$, where P is the tension, P_{init} is an initial tension, t is time, and A is the amplitude of the redeveloped tension. Tension-pCa curves were fit to the Hill equation in the form $P = P_o/(1 + 10^{(-nH(pK - pCa))})$, where P_o is the maximum tension developed, n_H is the Hill coefficient, and pK is the pCa at 0.5 P_o.

Data are presented as mean ± SEM, unless otherwise indicated. A paired Student's two-tailed t-test was used as a test of significance between two groups of data and a pairwise ANOVA for multiple data sets. The confidence level was set at $p < 0.05$.

RESULTS

Effect of nucleotide on Ca^{2+}-activated tension and stiffness

CTP proved to be an adequate substrate for tension production, as reported by other investigators (e.g., Pate et al., 1993). Fig. 1 shows an example of several activations in a single fiber, using either ATP or CTP as the substrate for tension production. As can be seen in this example, the effects of the nucleotide exchange were fully reversible. In rabbit skinned psoas fibers, CTP produced 94 ± 1% (n = 18) of the maximum Ca^{2+}-activated tension produced by ATP (80 ± 6 kN/m^2, n = 18). Tension-pCa curves were constructed (Fig. 2) to show that the Ca^{2+}-activated tension response had indeed saturated. To determine whether the decreased tension seen with CTP was due to a decrease in the force per cross-bridge interaction or a decrease in the number of interactions, the instantaneous stiffness of the fiber was also measured. Instantaneous stiffness provides an estimate of the number of strongly bound cross-bridges under the assumptions that the stiffness per strongly bound cross-bridge is a constant and that the myofilaments are not compliant (Huxley and Simmons, 1972). Although the latter assumption has come into question recently (Huxley et al., 1994; Wakabayashi et al., 1994), because the sarcomere length was kept constant here any effects of myofilament compliance are likely to also be constant and, therefore, should not produce any qualitative changes in the results. At maximum Ca^{2+} activation the instantaneous stiffness in psoas fibers was observed to be less affected than tension by the nucleotide exchange, increasing with CTP as substrate to 106 ± 2% (n = 6) of the ATP stiffness. This result indicates that there was a small increase in the number of strongly attached cross-bridges when CTP replaced ATP. As the Ca^{2+}-activated tension was varied over a wide range, the instantaneous stiffness varied in a nearly linear manner (Fig. 3). The slope of the relative tension versus relative stiffness curves decreased significantly from 1.01 ± 0.01 to 0.91 ± 0.01 (n = 6) when ATP was replaced with CTP.

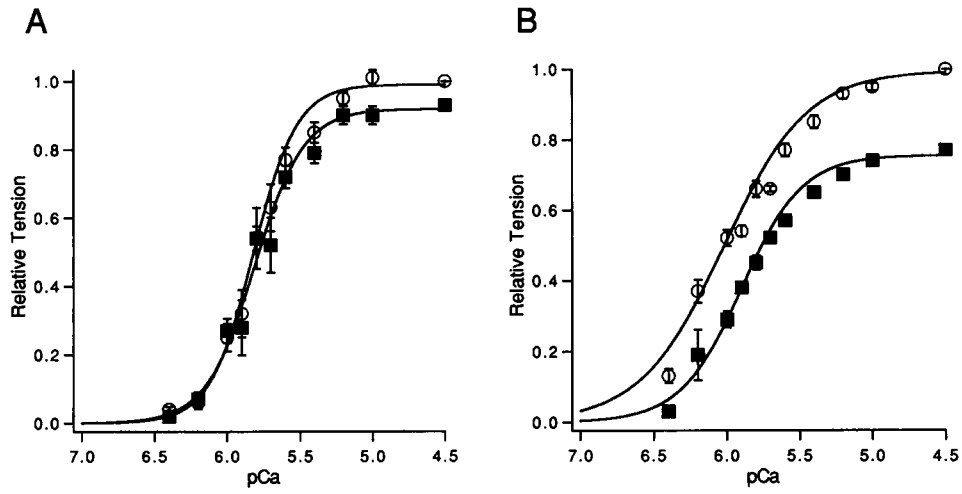

FIGURE 2 Summary of tension-pCa curves from single skinned fibers of rabbit psoas (*A*) and rat soleus (*B*) muscle. Tension produced by ATP (○)- and CTP (■)-containing solutions is normalized to the tension produced in the same fiber by ATP at pCa 4.5. Data were collected at 15°C. Solid lines represent the Hill equation fit to the data. (*A*) ATP: n_H = 2.88 ± 0.26, pCa_{50} = 5.82 ± 0.03; CTP: n_H = 2.57 ± 0.16, pCa_{50} = 5.81 ± 0.03; n = 11 fibers. (*B*) ATP: n_H = 1.51 ± 0.06, pCa_{50} = 6.02 ± 0.03; CTP: n_H = 2.06 ± 0.04, pCa_{50} = 5.90 ± 0.01; n = 12 fibers. Values are shown as mean ± SEM.

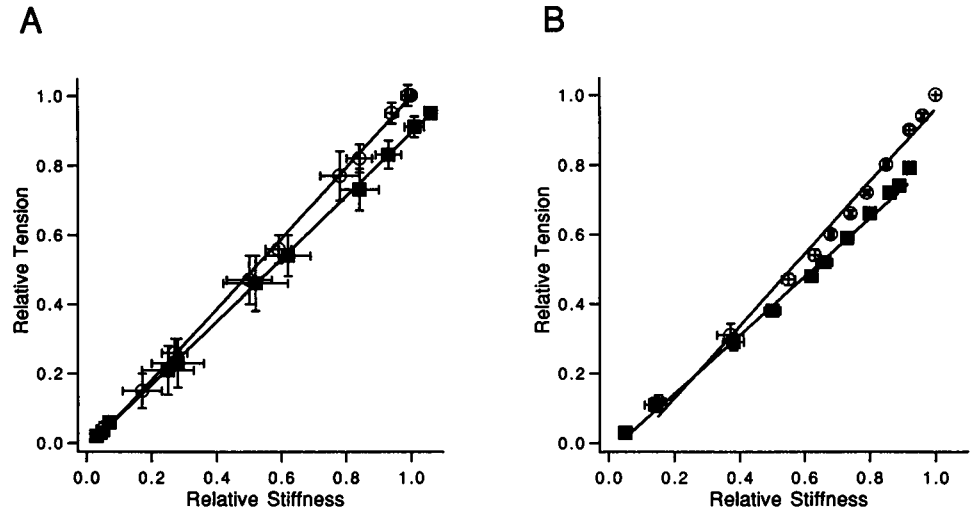

FIGURE 3 Summary of tension-stiffness relationship in single skinned fibers from rabbit psoas (*A*) and rat soleus (*B*) at 15°C. Tension and stiffness produced by ATP (○) and CTP (■) are normalized to the tension and stiffness produced by ATP at pCa 4.5 in the same fiber. Lines are linear regressions to the data. Values are shown as mean ± SEM.

Thus, the decreased tension observed with CTP must be due to either a decrease in the force produced per cross-bridge interaction or, alternatively, to a redistribution of strongly attached states (see Discussion).

Slow soleus fibers were more affected by the change in nucleotide, with CTP producing 77 ± 1% (n = 10) of the maximum Ca^{2+}-activated tension generated by ATP (176 ± 17 kN/m^2, n = 10; Fig. 2). Furthermore, the maximum stiffness in soleus fibers decreased with CTP to 92 ± 1% (n = 6) of the maximum ATP stiffness. This is in contrast to the small increase in fiber stiffness seen in psoas fibers. Thus, there was an important fiber type difference in the effect of nucleotide exchange on fiber stiffness. Soleus fibers also showed a decrease in the slope of the relative tension-stiffness relationship from 0.99 ± 0.01 with ATP to 0.84 ± 0.01 (n = 6) with CTP. Thus, the total force per cross-bridge interaction in both fiber types appeared to decrease with the nucleotide exchange, with the effect being greater in soleus fibers. Because both stiffness and the slope of the tension-stiffness curve decreased, it would appear that the decreased tension in slow fibers in the presence of CTP is a result of both a decreased number of strong interactions and a reduction in the total force produced per cross-bridge interaction. However, because stiffness decreased by a lesser extent than maximum tension, it would appear that the decreased tension is predominantly due to a decrease in the average force per cross-bridge interaction.

Closer examination of the tension-pCa and stiffness-pCa plots (Figs. 2 and 4) revealed additional muscle lineage differences in the effects of the nucleotide exchange. The psoas fibers exhibited no significant changes in n_H and only a small but significant (0.05 ± 0.01 pCa units; n = 6) leftward shift in the pCa_{50} of the stiffness-pCa plot with the change in nucleotide triphosphate. Changes in the pCa_{50} of the tension-pCa plot were not significant. Interestingly, in soleus fibers replacing ATP with CTP produced a substantial increase in the Hill coefficient (n_H) for both tension (from 1.51 ± 0.06 to 2.06 ± 0.04; n = 12) and stiffness (from 1.52 ± 0.09 to 2.25 ± 0.06; n = 6). Moreover, the pCa_{50} of both the tension-pCa and stiffness-pCa plots

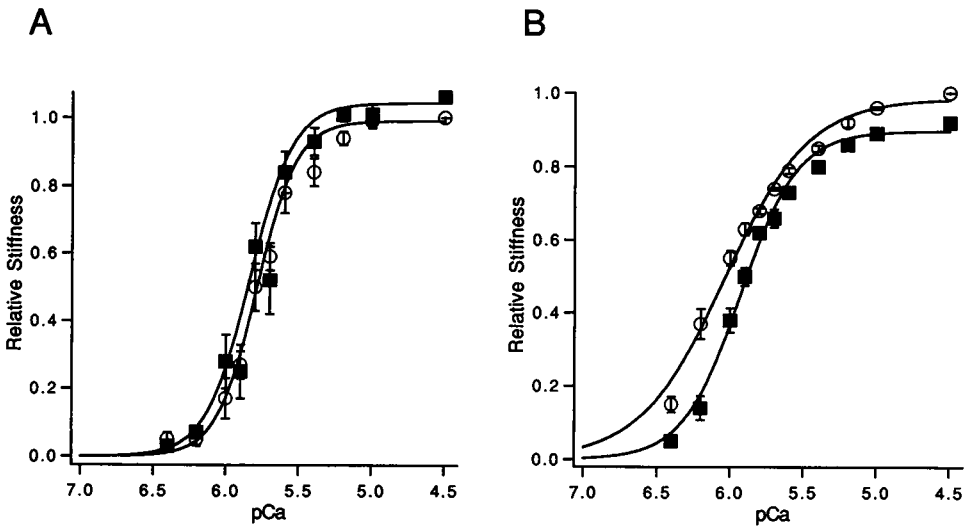

FIGURE 4 Summary of stiffness-pCa curves from rabbit psoas (*A*) and rat soleus (*B*) at 15°C. Stiffness produced by ATP (○)- and CTP (■)-containing solutions are normalized to the stiffness produced in the same fiber by ATP at pCa 4.5. Solid lines represent the Hill equation fit to the data. (*A*) ATP: n_H = 3.21 ± 0.40, pCa_{50} = 5.79 ± 0.04; CTP: n_H = 2.94 ± 0.13, pCa_{50} = 5.84 ± 0.04; n = 6 fibers. (*B*) ATP: n_H = 1.52 ± 0.09, pCa_{50} = 6.04 ± 0.02; CTP: n_H = 2.25 ± 0.06, pCa_{50} = 5.93 ± 0.02; n = 6 fibers. Values are shown as mean ± SEM.

shifted rightward (0.12 ± 0.02 (n = 12) and 0.11 ± 0.01 pCa units (n = 6), respectively) when CTP replaced ATP as the substrate. Overall, changes in the tension and stiffness-pCa curves produced by CTP caused the soleus fibers to more closely resemble the psoas fiber results, which exhibited a n_H of 2.88 ± 0.26 (n = 11) and 3.21 ± 0.40 (n = 6) for ATP-generated tension and stiffness, respectively (Figs. 2 and 4).

Rate of tension redevelopment

To determine the effects of CTP on the kinetics of tension production, the rate of tension redevelopment, k_{tr}, after a rapid shortening and restretch was determined (Brenner and Eisenberg, 1986). An example of the k_{tr} protocol is shown in Fig. 5. The maximum Ca^{2+}-activated k_{tr} generated by ATP for psoas fibers observed here at 15°C (11.7 ± 0.8 s^{-1}; n = 6) is somewhat slower than previously reported values for rat fast-twitch superior vastus lateralis fibers under similar experimental conditions (22.9 ± 0.5 s^{-1} at 15°C; Metzger and Moss, 1990). This difference is probably an interspecies difference in the rate of tension redevelopment. In psoas fibers at maximum Ca^{2+}-activated tension (pCa 4.5), k_{tr} increased from 11.7 ± 0.8 to 16.6 ± 0.9 s^{-1} (n = 6) when CTP was substituted for ATP. These values were obtained while controlling the sarcomere length by keeping the position of the first-order diffraction pattern constant. It is conceivable that the increased k_{tr} produced by CTP leads to a different step in the cross-bridge cycle becoming rate limiting. Because k_{tr} has been shown to be Ca^{2+} sensitive (Brenner, 1988; Metzger et al., 1989), the rate-limiting step of k_{tr} is likely to be closely coupled to a Ca^{2+}-sensitive step in the cross-bridge cycle. Thus, if replacing ATP with CTP causes a different kinetic step to become rate limiting, the Ca^{2+} sensitivity of k_{tr} could be altered. This point was investigated by constructing k_{tr}-pCa plots as shown in Fig. 6. These plots indicate that the k_{tr}-pCa relationship is not greatly altered by the change in nucleotide. In psoas fibers the only significant change in the Hill equation fit to the k_{tr}-pCa data was a small (0.12 ± 0.03 pCa units; n = 5) leftward shift in the pCa_{50} when CTP was used.

At maximum Ca^{2+} levels, k_{tr} in soleus fibers increased from 4.9 ± 0.2 to 8.4 ± 0.3 s^{-1} (n = 6) with the substitution of ATP by CTP. Thus, the soleus fibers are more dramatically affected by the change in nucleotide than the psoas fibers. This large increase is apparent at all Ca^{2+} levels (Fig. 6). The Hill equation fit to the k_{tr}-pCa data showed no significant change in either the n_H or pCa_{50} upon nucleotide exchange. Thus, it is likely that k_{tr} is limited by the same Ca^{2+}-sensitive step in the presence of either nucleotide, although the rate of this transition is increased in the presence of CTP. In contrast, the tension-pCa plots of soleus fibers were highly dependent on the nucleotide used (Fig. 2). This indicates that the Ca^{2+} sensitivity of tension and k_{tr} are not closely coupled in these fibers.

Effect of nucleotide concentration

Because the apparent binding of CTP to myosin is ~20-fold less than that of ATP (White et al., 1993), it could be argued that some of the mechanical properties observed here are due to an incomplete saturation of the nucleotide-binding

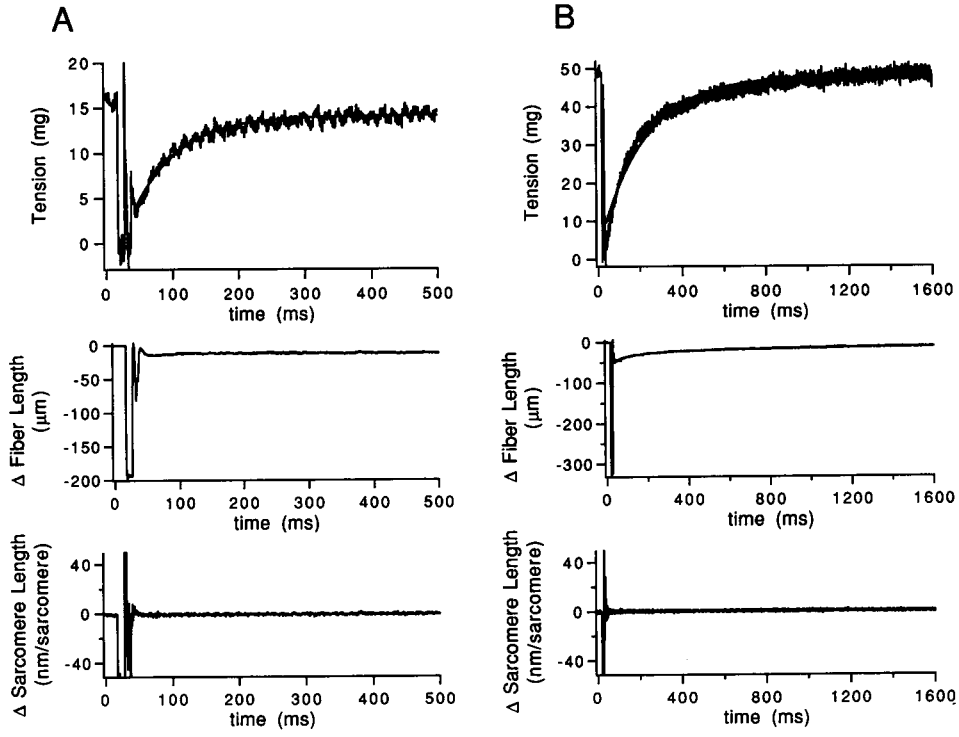

FIGURE 5 Representative traces of k_{tr} from rabbit psoas (*A*) and rat soleus (*B*) fibers at pCa 4.5. The top trace shows the tension in response to a rapid release and restretch of fiber length (*middle*) at 15°C. Changes in sarcomere length are shown at the bottom. Exponential fits used to derive k_{tr} are shown in the top traces.

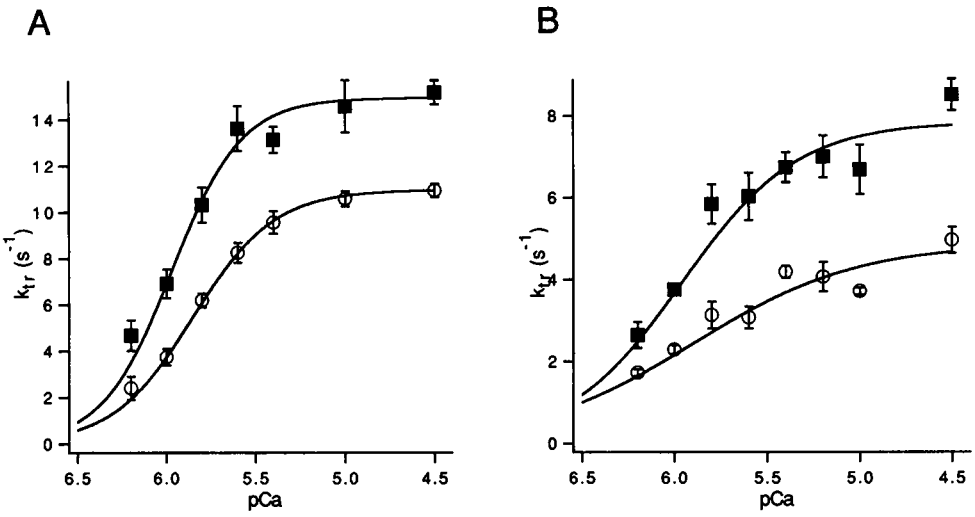

FIGURE 6 Summary of k_{tr}-pCa relationship for rabbit psoas (*A*) and rat soleus (*B*) at 15°C. ○, ATP data; ■, CTP data. Solid lines are Hill equation fits to the data. In this group of experiments, k_{tr} for the psoas fibers was collected in the absence of sarcomere length control. Thus the true isometric rate could be faster than observed here (Brenner and Eisenberg, 1986). At maximum Ca^{2+} activation, the k_{tr} measured here in psoas fibers without sarcomere length control was within 10% of the value obtained in psoas fibers with sarcomere length control. However, no qualitative differences were noted between k_{tr} traces obtained with and without sarcomere length control. In soleus fibers sarcomere length was kept constant at all tension levels under all conditions. Values are shown as mean ± SEM.

sites. This possibility was investigated by either reducing the MgATP concentration from 4 mM to 0.2 mM or increasing the MgCTP concentration from 4 mM to 10 mM. The reduction in ATP in soleus fibers increased the maximum Ca^{2+}-activated tension by a factor of 1.21 ± 0.02 (n = 4), while producing no significant effects on the shape or position of the tension-pCa curve. The effect of low [ATP] on the tension-pCa curve in psoas fibers was more severe. With 0.2 mM ATP, the pCa_{50} was shifted to the left by 0.3 pCa units and the n_H was increased from 2.7 ± 0.2 with 4 mM ATP to 11.9 ± 3.7 at 0.2 mM (n = 3). Furthermore, the decreased ATP in psoas fibers produced an increased tension of 1.14 ± 0.05 of the control value. These results are in contrast to the decreased force and Ca^{2+} sensitivity observed with 4 mM CTP (Fig. 2). Thus, the differences between 4 mM ATP and 4 mM CTP are not the result of incomplete saturation of the nucleotide-binding sites.

Increasing the CTP concentration from 4 mM to 10 mM lowered the maximum tension in soleus fibers from 0.81 ± 0.01 to 0.70 ± 0.01 (n = 4) of the tension produced by 4 mM ATP. This was accompanied by a small (~0.2 pCa units) increase in the observed rightward shift of the tension-pCa relation. Increasing CTP from 4 mM to 10 mM had similar effects on the tension-pCa curve in psoas fibers, producing a rightward shift in pCa_{50} from 6.00 ± 0.04 to 5.85 ± 0.04 (n = 4), while having no significant effect on n_H. The dramatic change in n_H of psoas fibers produced by low ATP would predict that even a small decrease in saturation would lead to an increase in n_H. The lack of a CTP concentration dependence on n_H is evidence that the small increase in n_H observed in psoas fibers when 4 mM CTP replaced 4 mM ATP is not entirely due to incomplete saturation. Indeed, in three of the four psoas fibers examined, the n_H was increased with increased [CTP]. As in soleus fibers, both concentrations of CTP produced less tension in psoas fibers than 4 mM ATP, with the decrease being smaller with 4 mM than with 10 mM (0.96 ± 0.01 and 0.79 ± 0.01 of the 4 mM ATP value, respectively). Thus, the lower concentration of either nucleotide resulted in more force and a greater Ca^{2+} sensitivity in both fiber types. However, the low ATP results are qualitatively different from the CTP results at either concentration.

Incomplete saturation of the nucleotide-binding sites would be expected to result in the formation of rigor bonds. The presence of rigor cross-bridges has been shown by other investigators to produce an increase in tension (Ferenczi et al., 1984; Pate et al., 1992) and a leftward shift in the tension-pCa relation (Godt, 1974). The additional decreases in tension and pCa_{50} with increasing CTP are, therefore, consistent with the presence of a small number of rigor cross-bridges at 4 mM CTP. However, the effects of these apparent rigor cross-bridges are small when compared to the differences observed by exchanging nucleotides. Therefore, the primary mechanism of the differences seen with nucleotide exchange cannot be due to incomplete nucleotide saturation of the fibers.

The effect of nucleotide concentration on k_{tr} was also investigated. In soleus fibers reduction of MgATP from 4 mM to 0.2 mM caused a fourfold reduction in k_{tr}. This effect is in opposition to the doubling of k_{tr} seen by exchanging 4 mM ATP with 4 mM CTP. Moreover, increasing the CTP to 10 mM caused a further increase (~20%) in k_{tr}. Increased CTP had no effect on k_{tr} in psoas fibers, whereas the reduction of ATP to 0.2 mM effectively eliminated the psoas k_{tr} response. These results are inconsistent with the effects of

CTP being due to insufficient saturation of the nucleotide binding sites.

Thus, because lowered ATP did not reproduce the observed effects of CTP, and the differences between 4 mM ATP and 4 mM CTP were not diminished by 10 mM CTP, but tended to be enhanced, it can be concluded that the effects of nucleotide exchange seen here are not due to differences in nucleotide saturation. Rather, the changes in mechanical properties of the fibers with nucleotide exchange represent nucleotide-dependent changes in contractility.

Effect of P_i

Tension production has been observed to be inhibited by P_i (Rüegg et al., 1971; Chase and Kushmerick, 1988; Godt and Nosek, 1989; Millar and Homsher, 1990), and the P_i release step of the actomyosin ATPase has been coupled to the transition from weak to strong binding (Hibberd et al., 1985). It was of interest, therefore, to determine how a change in nucleotide substrate would be affected by a variation in P_i concentration. Thus, in a separate study, solutions were made with 0–30 mM added P_i, and the effects on k_{tr} and tension were observed. In psoas fibers, using ATP as the substrate for tension production, k_{tr} increased from 10.5 ± 0.4 s^{-1} with nominal P_i to 20.0 ± 0.9 s^{-1} (n = 4) with 20 mM added P_i, as seen in Fig. 7. Beyond 20 mM added P_i, no further increases in k_{tr} were observed. Similarly, k_{tr} increased in the presence of CTP from 14.7 ± 0.4 s^{-1} to 29.9 ± 1.7 s^{-1} (n = 4) when the added P_i was increased from 0 to 20 mM. A hyperbolic fit to the k_{tr}-P_i data gave a k_D of 6.91 ± 1.48 mM and 4.97 ± 0.87 mM (n = 4) in the presence of ATP and CTP, respectively. This difference was not significant by Student's t-test. Tension, in contrast, was depressed in psoas fibers with increased P_i such that, in the presence of ATP, 20 mM added P_i decreased the maximum Ca^{2+}-activated tension to 45 ± 4% of the maximum tension produced at nominal P_i. This decrease is similar to previous measurements at 10°C (Millar and Homsher, 1990). With CTP the tension was depressed at high P_i to a similar amount (45 ± 3% at 20 mM added P_i). The k_D derived from the tension-P_i relationship, however, was significantly changed from 6.99 ± 1.26 mM in the presence of ATP to 3.03 ± 0.29 mM (n = 4) with CTP (Fig. 8).

In soleus fibers there was little change seen in k_{tr} with either nucleotide when P_i was added (Fig. 7), with values of 2.9 ± 0.1 s^{-1} and 3.1 ± 0.1 s^{-1} (n = 4) with ATP and 6.4 ± 0.3 s^{-1} and 4.7 ± 0.3 s^{-1} (n = 4) with CTP at 0 and 20 mM added P_i, respectively. Consequently, it was not possible to obtain a satisfactory hyperbolic fit to the data. The tension, in contrast, was depressed significantly, although to a lesser extent than observed in psoas fibers (Fig. 8). At 20 mM added P_i the tension was 61 ± 2% and 56 ± 1% (n = 4) of the control tension values for ATP and CTP, respectively. The hyperbolic fit to the tension decrease gave similar values for k_D of 3.1 ± 0.45 mM and 2.37 ± 0.30 mM (n = 4) in the presence of ATP and CTP, respectively. Thus there is a significant fiber type difference in the response to added P_i such that in soleus fibers there is no striking change in k_{tr} with added P_i, whereas in psoas fibers k_{tr} is greatly increased. Moreover, in soleus the tension-P_i relation is not significantly affected by a change in nucle-

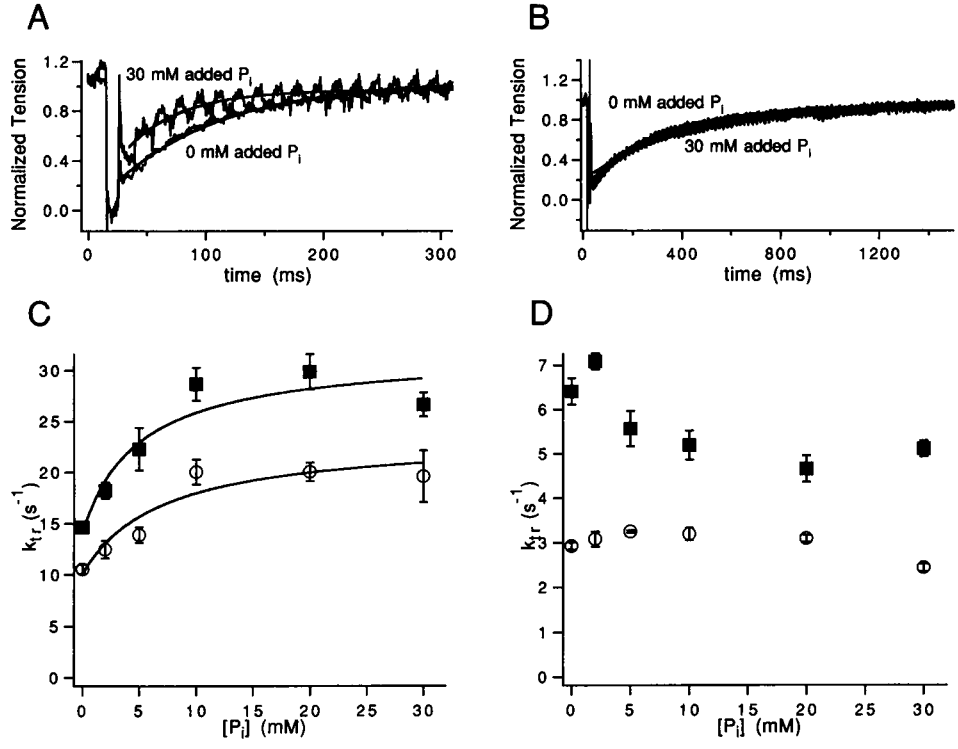

FIGURE 7 Effect of P_i concentration on k_{tr} in rabbit psoas (A, C) and rat soleus (B, D) at pH 4.5. Examples of k_{tr} traces with 0 and 30 mM added P_i are superimposed in A and B. Summaries of k_{tr} versus P_i concentration are shown in C and D. ○, ATP data; ■, CTP data. Data were collected at 15°C. Solid lines in C are hyperbolic fits to the data. It was not possible to fit the data in D by this equation. In this group of experiments, k_{tr} for psoas fibers was collected in the absence of sarcomere length control. As noted earlier in the legend to Fig. 6, no qualitative differences were seen between k_{tr} collected with and without sarcomere length control. In soleus fibers sarcomere length was kept constant at all tension levels under all conditions. Values are shown as mean ± SEM.

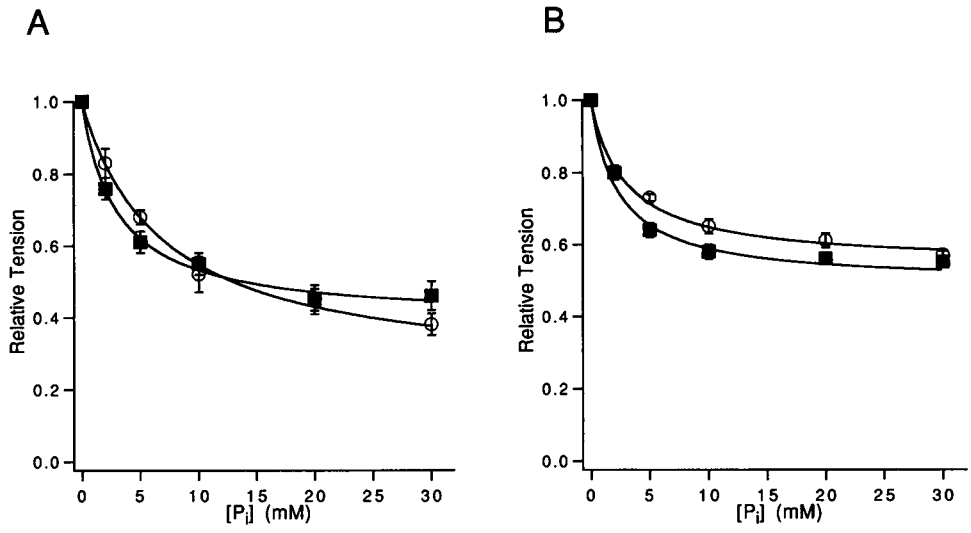

FIGURE 8 Summary of the effect of P_i concentration on maximum Ca^{2+}-activated tension in single skinned fibers from rabbit psoas (*A*) and rat soleus (*B*) at 15°C. ○, ATP data; ■, CTP data. Data are normalized to the value with 0 added P_i in the same fiber with the same nucleotide. Solid lines are hyperbolic fits to the data. Values are shown as mean ± SEM.

otide, whereas psoas fibers display a significant nucleotide-dependent change in the k_D derived from the tension-P_i relation.

DISCUSSION

The goal of this study was to elucidate differences in the mechanics of fast and slow skeletal muscle fibers by altering the chemistry of the mechanoenzyme through the use of the ATP analog CTP. The results with CTP were, in general, qualitatively similar in both fast and slow muscle fibers. However, there were important fiber type differences, particularly with regard to the tension- and stiffness-pCa relations and with regard to the effects of P_i on tension and k_{tr}. Quantitatively, the soleus fibers usually showed larger changes, and these changes were generally in the direction of becoming more like the psoas response. Thus, in most cases, the differences in contractile mechanics between fiber types were lessened in the presence of CTP.

Effect of nucleotide on Ca^{2+}-activated tension and stiffness

At maximum Ca^{2+} activation, CTP produced less tension when compared to the tension generated by ATP in both fiber types, although the effect was more pronounced in the soleus fibers. A decrease in maximum tension could be caused by a decrease in the number of force-producing cross-bridges or a decrease in the force produced by each cross-bridge interaction. By assuming that filament compliance and the stiffness of bound cross-bridges are unchanged by the change in nucleotide, instantaneous stiffness can be used as a measure of the relative number of bound cross-bridges (Huxley and Simmons, 1972). Thus, a decrease in the ratio of tension to instantaneous stiffness (Fig. 3) indicates an apparent decrease in the force per cross-bridge interaction. In psoas fibers the decrease in maximum Ca^{2+}-activated tension when ATP was replaced by CTP was accompanied by an increase in the instantaneous stiffness. Thus, there was an increase in the number of bound cross-bridges and a decrease in the apparent force per cross-bridge interaction. Under these same conditions, soleus fibers showed a decrease in the instantaneous stiffness, indicating a decrease in the number of bound cross-bridges. However, as with psoas fibers, the stiffness decrease was less than the decrease in tension. Thus, there is a decrease in the apparent force per cross-bridge interaction in soleus fibers as well. Because the tension-stiffness ratio depression was greater in soleus fibers than in psoas fibers, it follows that the larger decrease in maximum Ca^{2+}-activated tension observed in soleus fibers when CTP replaced ATP was due to greater decreases in both the number of bound cross-bridges and the force per cross-bridge interaction.

Given that there are likely to be several strongly bound states, which may bear different amounts of tension (Kawai and Zhao, 1993; Dantzig et al., 1992), there are two possible mechanisms for an apparent decrease in the force per cross-bridge interaction. First, the tension produced by one or more of the strongly bound states could be reduced. Because tension production is expected to be produced by specific conformational changes in the cross-bridge, this mechanism requires that these changes be sensitive to the nucleotide bound. Alternatively, there could be a change in the distribution of strongly bound states such that a greater proportion are in low force-producing states. Although this mechanism does not require any change in the individual force-producing states, it does require that there be a change in the kinetics of the transitions between them. The experiments reported here are unable to distinguish between these two mechanisms.

Differences in the effect of the nucleotide exchange on psoas and soleus fibers were also observed at submaximum Ca^{2+} levels. These differences are most easily seen by examining changes in the tension-pCa relationship (Fig. 2). The shape of the tension-pCa curve, as described by n_H, is

usually interpreted as indicative of the amount of cooperativity in activation of the thin filament. Thus, the high value of n_H observed in skinned fibers is evidence of cooperative interactions either within or between the thin-filament functional units consisting of one tropomyosin-troponin regulatory complex and its associated seven actin monomers (Bremel and Weber, 1972; Brandt et al., 1984; Moss et al., 1985). Inasmuch as a change in nucleotide is expected to affect only the cross-bridges and not the thin-filament regulatory proteins directly, the substantial increase in n_H when CTP replaced ATP in soleus fibers seems to require that there be cross-bridge interactions with the thin filament that affect interactions among the proteins of the thin-filament functional units. In view of the fact that solutions were made assuming the stability constants of CTP and ATP were identical (Pate et al., 1993), it might be argued that the changes in the tension-pCa relationship are due to small differences in the [Ca^{2+}] and not a difference in the Ca^{2+}-activation properties of the fibers. However, a small difference in the [Ca^{2+}] would not produce changes in n_H. Rather, it would produce a small shift in pCa_{50}, such as was observed in the stiffness-pCa curve of psoas and both stiffness-pCa and tension-pCa curves in soleus when CTP was substituted for ATP. Thus, the significantly increased n_H observed for both tension and stiffness in slow fibers must represent a real change in the Ca^{2+}-activation properties of slow fibers with CTP-bound cross-bridges. Moreover, because the same solutions were used for both fiber types and the pCa_{50} shift in the stiffness-pCa curve is to the right in slow fibers and to the left in fast fibers, it can be concluded that there is a change in the Ca^{2+} sensitivity of at least one of the fiber types due to the change in nucleotide.

In the presence of ATP, the tension-pCa curves of psoas fibers exhibited a larger n_H than is seen in soleus fibers. This indicates that the Ca^{2+} activation of fast fibers is more highly cooperative than in slow fibers, in agreement with earlier studies (Kerrick et al., 1976; Metzger and Moss, 1987). Partial extraction of TnC leads to a reduction in the cooperativity of Ca^{2+} activation, strongly suggesting that the cooperativity is due to interactions between adjacent functional groups (Brandt et al., 1984; Moss et al., 1985). When CTP was used as the substrate for tension production in soleus fibers there was an apparent increase in the cooperativity. If cooperativity is solely the result of near-neighbor interactions, this result would seem to suggest that the presence of CTP strengthens the interfunctional unit interactions of the thin filament in slow fibers. A simpler explanation would be that part of the higher degree of cooperativity observed in fast fibers when compared to slow may be due to a cooperative cross-bridge effect within a functional unit independent of any interactions between neighboring functional units along the thin filament. It is expected that this type of cooperativity would be unaffected by TnC extraction. Furthermore, the change in the steady-state Ca^{2+} response with a change in nucleotide could be interpreted as evidence of a Ca^{2+}-sensitive step in the cross-bridge cycle that is affected by the bound nucleotide.

It is unclear at present how the nucleotide bound to myosin can produce changes in the Ca^{2+}-activation properties of the fiber. In these experiments the fibers were activated for sufficiently long periods for Ca^{2+} to become bound to the myosin regulatory light chains (Robertson et al., 1981), which has been proposed to produce changes in the cross-bridge kinetics (Metzger and Moss, 1992). However, the binding of Ca^{2+} to the light chains should occur with either nucleotide and therefore is not sufficient to explain the changes in Ca^{2+} activation seen here. Alternatively, it is possible that the binding of myosin to actin causes changes in the actin monomer, which are then transmitted to the regulatory proteins. Studies with the peptide phalloidin, which binds specifically to actin and strengthens the interaction between actin monomers (Estes et al., 1981), have been reported to alter the Ca^{2+} sensitivity of the thin filament (e.g., Bukatina et al., 1995), suggesting that an actin-mediated mechanism is feasible. However, the two fiber types used here contain the same actin isoform (Gros and Buckingham, 1989). Therefore, an actin-mediated pathway would require that the actomyosin interaction produce conformational changes in actin that are specific to the myosin isoform and the nucleotide bound to myosin.

Another mechanism for producing changes in the cooperativity of activation involves an interaction between myosin and the thin-filament regulatory proteins directly, particularly tropomyosin. This type of interaction provides a more direct pathway between myosin and the Ca^{2+}-binding sites on troponin C. In this mechanism the binding of myosin in certain specific cross-bridge states serves to activate the thin filament directly. Evidence for this type of pathway is found in the classic studies by Bremel and Weber (1972), which showed that the presence of rigor bridges could both increase the Ca^{2+} affinity of the thin filament and cause partial activation of the thin filament, even in the absence of Ca^{2+}. However, although the CTP actomyosin dissociation constant is only 5% of the ATP dissociation constant (White et al., 1993), with 4 mM nucleotide triphosphate the actomyosin dissociation is still a rapid step with either nucleotide. Furthermore, the presence of rigor bonds has been shown to produce an increase in maximum tension (Ferenczi et al., 1984; Pate et al., 1992) and a marked leftward shift in the tension-pCa relation (Godt, 1974). In contrast, 4 mM CTP decreased tension and shifted the tension-pCa curve to the right. Therefore, the possible formation of rigor bonds with 4 mM CTP cannot account for the nucleotide-dependent changes in contractility observed here. This does not exclude, however, the possibility that other strongly bound states also influence the Ca^{2+} affinity of the thin filament. Indeed, by using fluorescently labeled TnC, there is evidence that cycling cross-bridges not only are capable of increasing the Ca^{2+} affinity of TnC, but do so more effectively than rigor cross-bridges (Güth and Potter, 1987). Thus, there appear to be cycling cross-bridge states that can alter the Ca^{2+}-acti-

TABLE 1 Rate constants used to simulate k_{tr} by the model illustrated in Scheme 2

	Fast		Slow	
	ATP	CTP	ATP	CTP
k_{+2}	50	50	50	50
k_{-2}	10	10	10	10
k_{+3}	20	38	3.8	8
k_{-3}	5	5	0.6	2
k_{+4}	12	13	12	80
k_{-4}	44	48	20	300
k_{+5}	600	600	40	40
k_{-5}	8.6×10^4	2.0×10^5	1.3×10^4	1.7×10^4
k_{+6}	2	0.7	0.9	0.5

The forward transition rate constant of step x is abbreviated as k_{+x}, the reverse rate constant is designated k_{-x}, and $K_x = k_{+x}/k_{-x}$. Values for steps 1 and 2 are assigned as in Regnier et al. (1995). K_1 was set at 10^6. The value of K_5 was taken from the hyperbolic fit of the tension versus P_i plots (Fig. 8). Because step 5 is a rapid equilibrium, k_{+5} was set at 600 s^{-1} for fast fibers and was assumed to be 15 times slower for slow fibers (Millar and Homsher, 1992). The k_{-5} was then calculated from k_{+5} and the observed K_5. The k_{+6} was set following Regnier et al. (1995), with a slight modification to better fit the data, at 2 s^{-1} for ATP in fast fibers, and, because this step is assumed to be the rate-limiting step of high velocity, the value of k_{+6} for the remaining conditions was scaled by the ratio of V_{max} in the presence of ATP and CTP taken from earlier results (Wahr and Metzger, 1996). The values of k_{+3}, k_{-3}, k_{+4}, and k_{-4} were then varied to give the best fit to the k_{tr} data.

vation properties of the fiber. CTP may increase the ability of these cross-bridge states to alter the Ca^{2+} activation directly, or, by altering the kinetics, it may increase the proportion of cross-bridges in these states. Regardless of the mechanism, because the increased cooperativity was observed only for soleus fibers, it can be concluded that the thin-filament regulatory proteins in slow fibers are more sensitive to nucleotide-mediated changes in the cross-bridge states.

It has been reported that the stiffness of the thin filament is dependent on the nucleotide (ATP or ADP) bound to actin (Janmey et al., 1990). Although there is some dispute over these findings (Newman et al., 1993), this does raise the possibility that some of the effects of nucleotide exchange reported here could be unrelated to changes in the nucleotide bound to myosin, but instead are caused by an exchange of the nucleotide bound to actin. This would require that the nucleotide bound to actin be effectively replaced in the relatively short time (a few minutes) between the initial exchange of the solutions and the activation of the fiber. This is unlikely, however, because the tightly bound ADP normally formed during thin-filament formation appears to be essentially nonexchangeable once the filaments are formed (Martonosi et al., 1960; Newman et al., 1993). Moreover, even if significant exchange of the nucleotide bound to actin did occur, in vitro motility studies of F-actins prepared with a variety of bound nucleotides have not

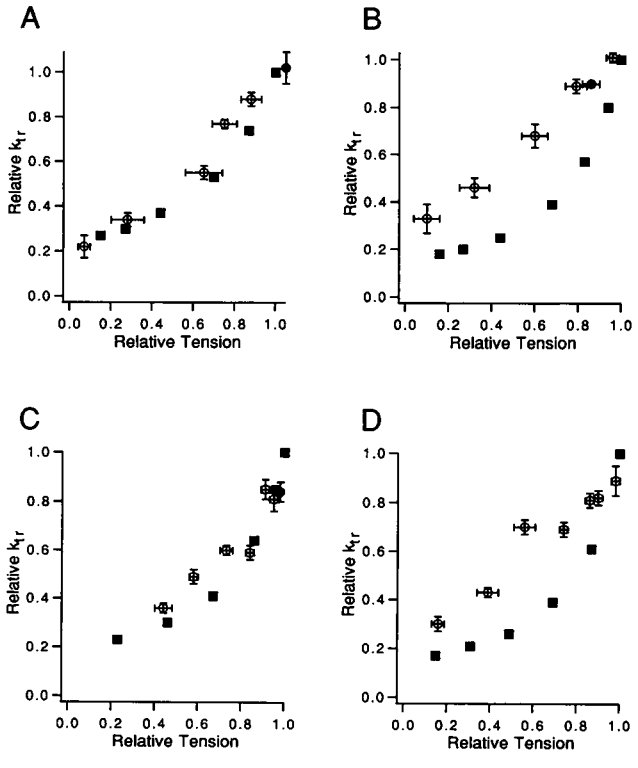

FIGURE 9 Modeled k_{tr} at different levels of Ca^{2+}-activated tension compared to data from psoas (*A*, *B*) and soleus (*C*, *D*) fibers in the presence of ATP (*A*, *C*) or CTP (*B*, *D*). ○, Data points; ■, fits to the model. Error bars on the data points represent SEM.

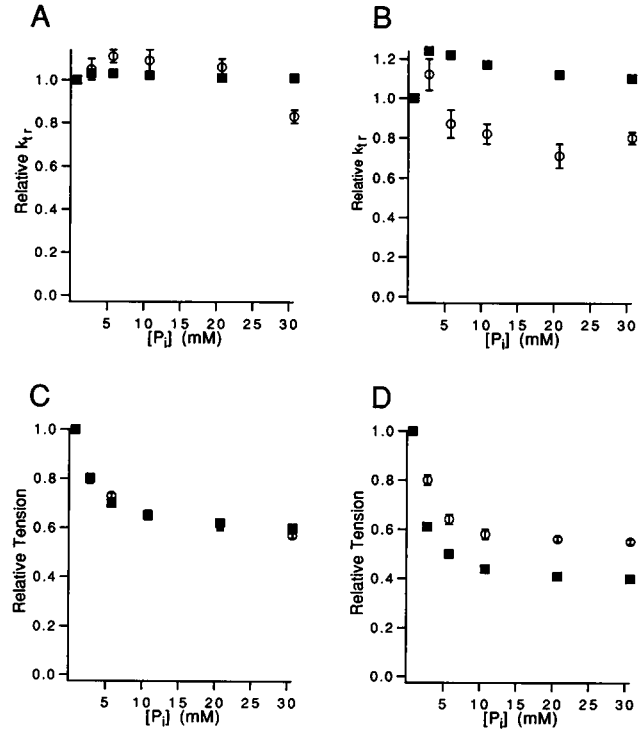

FIGURE 10 Modeled k_{tr} (*A*, *B*) and tension (*C*, *D*) at maximum Ca^{2+} and varied levels of P_i compared to data from soleus fibers in the presence of ATP (*A*, *C*) or CTP (*B*, *D*). ○, Data points; ■, fits to the model. Error bars on the data points represent SEM.

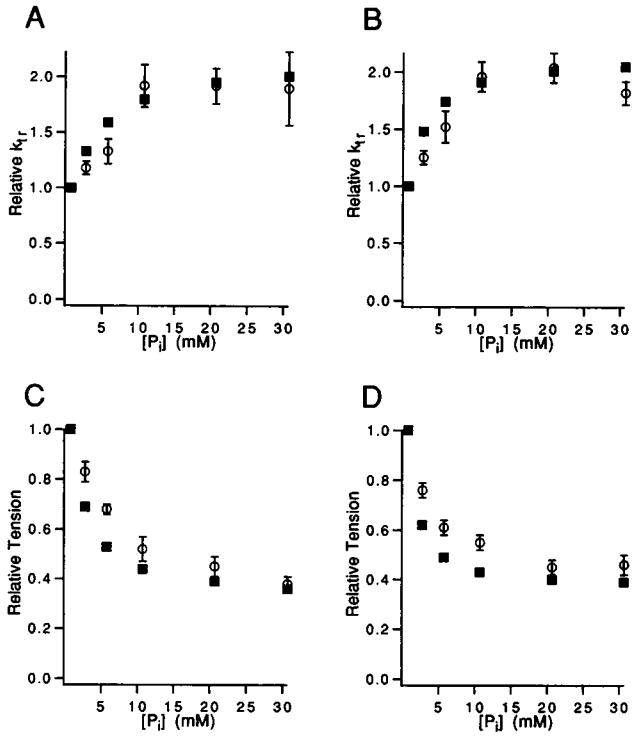

FIGURE 11 Modeled k_{tr} (*A*, *B*) and tension (*C*, *D*) at maximum Ca^{2+} and varied levels of P_i compared to data from psoas fibers in the presence of ATP (*A*, *C*) or CTP (*B*, *D*). ○, Data points; ■, fits to the model. Error bars on the data points represent SEM.

reported any significant alterations in the actomyosin interaction (Oishi and Sugi, 1994). This suggests that changes in the thin-filament nucleotide status are unlikely to be a major cause of alterations in the mechanics of contraction. Thus, it is reasonable to assume that the effects on mechanics due to nucleotide exchange are mediated by the cross-bridges.

Effect of nucleotide exchange on the rate of tension redevelopment

There is considerable debate about the role of the cross-bridges in activating the thin filament. There is evidence that Ca^{2+} binding to TnC is not sufficient to completely activate the thin filament, but that cross-bridge binding is also required (Hill et al., 1983; McKillop and Geeves, 1993; Lehman et al., 1996). It has been suggested, therefore, that the thin filament becomes deactivated by the cross-bridge disruption involved in making k_{tr} measurements (Millar and Homsher, 1990). Thus, k_{tr} would be limited by the rate at which cross-bridge binding reactivates the thin filament. However, deactivation of the thin filament would be expected to be accompanied by movement of the tropomyosin to its position in resting muscle. Movements of tropomyosin from the active to resting position appear to be too slow to cause significant thin-filament deactivation within the brief time required for the release and restretch of the k_{tr} measurement (Ishii and Lehrer, 1993). Furthermore, x-ray diffraction studies indicate that Ca^{2+} binding alone is sufficient to produce movement of the tropomyosin molecule (Kress et al., 1986). In addition, CTP increased k_{tr} significantly in both fiber types. Therefore, the results seen here favor k_{tr} being limited by transitions within the cross-bridge cycle and not by a thin-filament reactivation.

Both fiber types showed a k_{tr} dependence on Ca^{2+}. This would seem to indicate that k_{tr} is limited in both fiber types by a Ca^{2+}-sensitive step. Furthermore, the k_{tr}-pCa relationship was unaltered by a change in nucleotide. Because different steps of the cross-bridge cycle are unlikely to have the same Ca^{2+} dependency, this leads to the conclusion that the rate-limiting step of k_{tr} is the same Ca^{2+}-sensitive step with either nucleotide. Furthermore, assuming the cross-bridge cycle is similar for both fiber types, if Ca^{2+} has its effect on a specific transition in the cycle, this implies that both fiber types are limited by the same Ca^{2+}-sensitive step (but see discussion below and Appendix).

Effect of P_i on tension and k_{tr}

In agreement with previous studies (e.g., Rüegg et al., 1971; Chase and Kushmerick, 1988; Godt and Nosek, 1989; Millar and Homsher, 1990), P_i decreased the maximum Ca^{2+}-activated tension significantly in both fiber types. There is good evidence (Hibberd et al., 1985; Millar and Homsher, 1990) that the force-generating transition is coupled to P_i release. Hence, it is interesting that P_i had very different effects on k_{tr} in fast and slow muscle fibers. In psoas fibers the decrease in maximum Ca^{2+}-activated tension seen with increased P_i was accompanied by an increase in k_{tr}, as would be predicted if the P_i release step were closely coupled to the rate-limiting step of k_{tr}. Further increases in P_i caused the effect to become saturated. This indicates that at high P_i a different, earlier step in the cross-bridge cycle limits k_{tr}. On the other hand, in soleus fibers there was no consistently observed increase in k_{tr} with added P_i, indicating that in this fiber type P_i release does not limit k_{tr}, but apparently follows an earlier rate-limiting transition. This is consistent with the results of caged P_i experiments, which indicate that the rate-limiting step of ATP turnover in slow fibers occurs earlier in the cross-bridge cycle than in fast fibers (Millar and Homsher, 1992). Thus, at maximum Ca^{2+} activation, there appears to be a muscle lineage difference as to which step is rate limiting for k_{tr}, at least at nominal P_i. This is in conflict with the conclusion from the k_{tr}-pCa results, which indicated that the same step was rate limiting in both fiber types. Reconciliation of this conflict requires either that different steps are Ca^{2+} sensitive in the different fiber types or that there are multiple Ca^{2+}-sensitive steps. Seeing that CTP caused k_{tr} to increase, it obviously increases the rate-limiting step, possibly to an extent that a different step in the cycle became rate limiting. If this were the case, a change in the k_{tr}-Ca^{2+} and k_{tr}-P_i relationships would be expected. However, no change was apparent in either fiber type, indicating that the rate-limiting step of k_{tr}

using ATP as the substrate remains rate limiting when CTP replaces ATP. To address this issue further, the k_{tr} data were fit to a published model incorporating specific steps of the cross-bridge cycle. The results of these fits are given in an appendix.

CONCLUSIONS

The cross-bridge cycle plays several important roles in determining the contractile mechanics of the fiber. By altering the cycle through the use of the ATP analog CTP, we have been able to alter not only kinetic parameters, such as k_{tr}, but also the Ca^{2+} activation characteristics. Interestingly, these changes served to decrease the differences between fiber types, primarily by causing slow fibers to more closely resemble fast fibers. Thus, it appears that differences in the nucleotide-binding region of myosin are capable of producing many of the differences between fast and slow fibers.

APPENDIX: MODELS OF CONTRACTION IN FAST AND SLOW MUSCLE

The data on the rate of tension redevelopment was fit to a six-state cross-bridge kinetic model (Scheme 2, where weak and strong cross-bridge states are indicated) proposed by Regnier et al. (1995) in an attempt to assign rates to specific cross-bridge transitions. This model was based on the earlier model of Millar and Homsher (1990) and includes a weak-to-weak transition that is sensitive to Ca^{2+} (step 3) before the weak-to-strong force-generating step 4. Although the psoas data could be fit by a simple three-state model, incorporating a Ca^{2+}-sensitive weak-to-strong transition, attempts to fit the soleus data to simpler models that did not include this separation of the Ca^{2+}-sensitive and force-producing steps were unsuccessful. Thus, because it was desired to compare derived rate constants from both fiber types, it was necessary to use this more complex model. Values for the rate constants, shown in Table 1, were taken from the literature, derived from the data, or varied to obtain the best fit. Tension and k_{tr} were determined from the model as in Regnier et al. (1995), and k_{+3} was assumed to be proportional to the $[Ca^{2+}]$. The k_{tr} values produced by the model are compared to the skinned fiber data in Figs. 9–11.

As can be seen in Figs. 9–11, the model gives a reasonably good fit to the skinned fiber data. Because the rates of steps 3 and 4 were used to fit the data, whereas the other steps are either derived directly from the data or assumed to be constant, these steps are of the greatest interest in terms of interpretation of the model. There are two main points to be inferred from Table 1. First, because the tension is produced by the AM′·ADP·P_i and AM′·ADP states, k_{tr} will be limited by transitions preceding these states. Therefore, it is apparent from the values in Table 1 that at high Ca^{2+} and nominal P_i that step +3 is the rate-limiting step in soleus, whereas in psoas it is step +4. These apparent rate-limiting steps correspond closely to the k_{tr} values observed in the fibers at low P_i (compare Table 1 and Fig. 5). Second, this difference in the rate-limiting step accounts for the differential effects of P_i in the two fiber types. As P_i increases, there is a shift in the equilibrium of the force-producing states toward the AM′·ADP·P_i state, which tends to drive step 4, the weak-to-strong force-generating step, in the reverse direction. Thus the tension is expected to decrease with increased P_i, as is observed in both fiber types. In fast muscle fibers the decrease in tension seen with increased $[P_i]$ is accompanied by an increase in k_{tr}, as would be predicted if step +4 were rate limiting for k_{tr}. Because this appears to be the rate-limiting transition in psoas, k_{tr} in psoas increases with added P_i until it approaches the rate of an earlier transition, which then becomes rate limiting. The value of k_{+3}, 20 s^{-1}, is in good agreement with the value of k_{tr} seen in psoas at high P_i and thus appears to be the rate-limiting step at high P_i. On the other hand, in slow muscle fibers the significant decrease in tension was not accompanied by an increase in k_{tr}, indicating that the rate-limiting step of k_{tr} occurs earlier in the cross-bridge cycle. Thus, in soleus, k_{+3}, which agrees with the value of k_{tr} seen in soleus, appears to be rate limiting at all P_i concentrations, and thus k_{tr} is unaffected by increased P_i.

$$\underset{\text{AM}}{1} \leftrightarrow \underset{\text{M.ATP}}{2} \leftrightarrow \underset{\text{A-M.ADP.P}_i}{3} \leftrightarrow \underset{\text{AM.ADP.P}_i}{4} \leftrightarrow \underset{\text{AM'.ADP.P}_i}{5} \leftrightarrow \underset{\text{AM'.ADP}}{6} \leftrightarrow \text{AM}$$

| weak binding states | strong binding states

SCHEME 2

In both fiber types the model requires an increase (~2-fold) in the putative Ca^{2+} sensitive weak-to-weak transition (k_{+3}) to fit the CTP results. In psoas fibers there is also a decrease in the bimolecular rate constant of P_i binding, k_{-5}. However, only small changes in the weak-to-strong transition (step 4) are necessary in psoas. In fact, an adequate fit to the data can be obtained in psoas without these small weak-to-strong changes by additional increases in k_{+3}. This is in stark contrast to the soleus data, which require large changes in the modeled weak-to-strong transition rates (k_{+4} and k_{-4}), but no change in k_{-5}. Thus the model indicates that k_{tr} is rate limited in the two fiber types by different steps in the cross-bridge cycle at nominal P_i, k_{+4} in psoas and k_{+3} in soleus. Furthermore, in agreement with the data, these steps limit k_{tr} with either nucleotide.

The above model correctly predicts the behavior of certain kinetic aspects of the data. The model does not, however, correctly predict the tension-pCa curves and is unable to predict the observed changes in fiber stiffness seen with the change in nucleotide. Thus, although useful, this model is not a fully accurate description of muscle contraction.

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