Calcium Regulation of Tension Redevelopment Kinetics with 2-Deoxy-ATP or Low [ATP] in Rabbit Skeletal Muscle

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ABSTRACT The correlation of acto-myosin ATPase rate with tension redevelopment kinetics (k_{tr}) was determined during Ca^{+2}-activated contractions of demembranated rabbit psoas muscle fibers; the ATPase rate was either increased or decreased relative to control by substitution of ATP (5.0 mM) with 2-deoxy-ATP (dATP) (5.0 mM) or by lowering [ATP] to 0.5 mM, respectively. The activation dependence of k_{tr} and unloaded shortening velocity (V_u) was measured with each substrate. With 5.0 mM ATP, V_u depended linearly on tension (P), whereas k_{tr} exhibited a nonlinear dependence on P, being relatively independent of P at submaximum levels and rising steeply at $P > 0.6–0.7$ of maximum tension (P_o). With dATP, V_u was 25% greater than control at P_o and was elevated at all $P > 0.15P_o$, whereas P_o was unchanged. Furthermore, the Ca^{+2} sensitivity of both k_{tr} and P increased, such that the dependence of k_{tr} on P was not significantly different from control, despite an elevation of V_u and maximal k_{tr}. In contrast, lowering [ATP] caused a slight (8%) elevation of P_o, no change in the Ca^{+2} sensitivity of P, and a decrease in V_u at all P. Moreover, k_{tr} was decreased relative to control at $P > 0.75P_o$, but was elevated at $P < 0.75P_o$. These data demonstrate that the cross-bridge cycling rate dominates k_{tr} at maximum but not submaximum levels of Ca^{2+} activation.

INTRODUCTION

Tension development in muscle results from cyclic interactions between myosin cross-bridges and actin and this interaction is regulated by the troponin-tropomyosin complex on the thin filament. The initial step in tension generation is Ca^{2+} binding to the thin filament regulatory protein troponin C (TnC) (Chalovich, 1992; Farah and Reinach, 1995; Grabarek et al., 1992; Tobacman, 1996). In chemically skinned fibers, where the level of activating Ca^{2+} can be controlled, both the steady-state level of tension (P) and the rate of tension redevelopment (k_{tr}) after a release-restretch cycle are Ca^{2+}-sensitive (Brenner, 1988; Brenner and Eisenberg, 1986). The relationship between P and k_{tr} with Ca^{2+} activation is curvilinear in skeletal muscle fibers (Brenner, 1988; Chase et al., 1994; Metzger et al., 1989; Metzger and Moss, 1991, 1992; Regnier et al., 1996; Sweeney and Stull, 1990). At 10°C, k_{tr} is slow ($\sim$1–2 s^{-1}) and Ca^{2+}-independent unless P is $>$ 50% of maximally activated tension (P_o). Thereafter, k_{tr} increases 10–15-fold as P increases to P_o at high levels of Ca^{2+} activation. This relationship indicates that Ca^{2+} regulation of tension development kinetics does not result simply from cross-bridge recruitment. Instead, because k_{tr} is thought to report the rate of cross-bridge transition from detached or weakly attached states to tension-generating states, the strong Ca^{2+} dependence of k_{tr} implies that Ca^{2+} controls the rate-limiting process in tension development (Brenner, 1988; Brenner and Eisenberg, 1986).

The mechanism by which Ca^{2+} controls the rate-limiting process in tension development is unclear. Metzger and Moss (1991) found that k_{tr} varies with [Ca^{2+}] rather than the number of activated thin filaments (as modified by extracting TnC), and interpreted their results as evidence for a direct effect of Ca^{2+} on an apparent rate constant that limits formation of strongly bound, tension-bearing cross-bridge states. Using a two-state model, it has been suggested that the forward rate constant of tension generation is the Ca^{2+}-dependent process (Brenner, 1988; Sweeney and Stull, 1990). This model predicts a unique relationship between P and k_{tr} that does not directly account for changes in thin filament Ca^{2+}-binding properties. However, when the Ca^{2+}-binding kinetics of TnC are altered, the P-k_{tr} relationship can be greatly affected. For example, replacement of native TnC with an activated form of TnC (aTnC) completely abolishes the Ca^{2+} sensitivity of k_{tr}, and cardiac forms of TnC decrease the Ca^{2+} sensitivity of k_{tr} (Chase et al., 1994). Furthermore, we have recently shown that calmidazolium (CDZ), a compound that specifically reduces the rate of Ca^{2+} dissociation from TnC (El-Saleh and Solaro, 1987; Johnson et al., 1994; Wahr et al., 1993), elevates k_{tr} during submaximum Ca^{2+} activations compared to tension-matched control measurements. These studies indicate a role for thin filament activation kinetics in regulating k_{tr} and argue against Ca^{2+} acting solely on cross-bridges to control the rate of tension generation.

The kinetic properties of myosin have also been shown to influence the rate of tension development in skeletal muscle. Metzger and Moss (1990) have shown that k_{tr} correlates with myosin isoform in maximally activated fast and slow mammalian skeletal muscle fibers. Furthermore, when myosin is modified by removal or phosphorylation of light chain 2 (LC_2), the Ca^{2+} sensitivities of both P and k_{tr} are

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increased (Metzger et al., 1989; Metzger and Moss, 1992; Sweeney and Stull, 1990). However, when these effects are characterized by the P-k_{tr} relationship, there is only a minor increase in k_{tr} at submaximum P, with no effect at P_o.

In maximally Ca^{2+}-activated fibers, substrate conditions that alter the rate of cross-bridge cycling bring about correlated changes in k_{tr}. Regnier et al. (1998) and Regnier and Homsher (1998) have shown that in maximally Ca^{2+}-activated fibers, replacement of ATP with 2-deoxy-ATP (dATP) increased both unloaded shortening velocity (V_u) and k_{tr}. This occurred with little or no effect on P_o. Measurements from caged P_i experiments indicated that dATP increases the rates of the tension-generating step and the release of dADP from the cross-bridge, in both isometric and shortening contractions. In solution, rates of nucleotide hydrolysis by acto-HMM (V_{max}) and F-actin motility in vitro (V_f) were also increased, but dATP binding to and cleavage by myosin were not different from those of ATP. Therefore it was suggested that the rate of cross-bridge cycling is faster with dATP because of increased rates at both the beginning and end of the cross-bridge power stroke. Conversely, in maximally Ca^{2+} activated fibers, reducing the [ATP] from 5 mM to $\leq$0.5 mM slows V_u, V_f, V_{max}, and k_{tr} (Chase and Kushmerick, 1995; Regnier and Homsher, 1998; Regnier et al., 1998), suggesting that the rate of cross-bridge cycling is slowed by lowering [ATP] because of slower cross-bridge release at the end of the power stroke. These types of experiments show that altering substrate conditions can be used to systematically increase or decrease k_{tr} in maximally Ca^{2+}-activated fibers.

Whereas cross-bridge transition rates determine k_{tr} in maximally Ca^{2+} activated fibers, it is not clear whether k_{tr} is limited by cross-bridge or thin filament kinetics during submaximum Ca^{2+} activations. To determine the extent to which cross-bridge kinetics influence the rate of tension development during submaximum Ca^{2+} activations, we compared measurements of P, V_u, and k_{tr} when 5 mM ATP was reduced to 0.5 mM ATP or replaced with 5 mM dATP. These conditions were chosen because they cause an ~30% decrease (with 0.5 mM ATP) or increase (with 5 mM dATP) in acto-HMM NTPase and V_u in maximally Ca^{2+} activated fibers, while having little or no effect on P_o (Regnier et al., 1998). Compared to tension-matched control measurements in the same fiber, dATP increased V_u for all Ca^{2+} activation levels, resulting in $P > 0.25P_o$. Furthermore, dATP increased the Ca^{2+} sensitivity of both P and k_{tr}. However, P_o was not affected by dATP, and k_{tr} was increased from tension-matched controls only when P was near maximum (i.e., $>0.75P_o$). In contrast, reducing the [ATP] from 5 mM to 0.5 mM slowed V_u for most Ca^{2+} activation levels, but had little or no effect on the Ca^{2+} sensitivity of P. Interestingly, the Ca^{2+} sensitivity of k_{tr} was decreased by reduction of [ATP] to 0.5 mM. This resulted from a small increase in k_{tr} at low levels of Ca^{2+} activation, coupled with a depression of k_{tr} at high levels of activation. The results presented here, when coupled with our earlier work showing that thin filament activation kinetics of skeletal muscle fibers can alter the Ca^{2+} dependence of k_{tr} (Chase et al., 1994; Regnier et al., 1996), suggest that 1) at low Ca^{2+} activation levels, k_{tr} is dominated by the kinetics of thin filament activation, and 2) at high Ca^{2+} activation levels k_{tr} is dominated by the kinetics of cross-bridge transitions. A preliminary report of this work was published previously (Regnier et al., 1997).

MATERIALS AND METHODS

Fiber preparation

Glycerinated segments of individual, fast fibers from rabbit psoas muscle were prepared according to the method of Chase and Kushmerick (1988). Isolated fiber segments were treated for 10 min with the nonionic detergent Triton X-100 (1%) in a 50% glycerol/relaxing (v:v) solution to remove residual membrane fragments. Fiber end compliance was minimized by regional microapplication of 1% gluteraldehyde (in H_2O) to chemically fix fiber segment ends (Chase and Kushmerick, 1988). After fixation of the ends, fiber segments were wrapped in aluminum foil T-clips for attachment to small wire hooks on the mechanical apparatus. A drop of silicone sealant was placed on the T-clip to further stabilize the attachment to the hook. Digital images of relaxed fibers and fibers during steady-state contractions were obtained at 0.32 μm/pixel (32$\times$ objective) or 2.56 μm/pixel (4$\times$ objective) with an XR-77 CCD camera (Sony, Japan), DT3155 frame grabber, and Global Lab Image software (Data Translation, Marlboro, MA). Fiber diameter and total fiber length (length of fiber segment between T-clips, including both unfixed and fixed fiber regions) were measured at the beginning of each experiment. The unfixed portion of fiber length (L_o) was determined at the conclusion of each experiment as previously described (Chase and Kushmerick, 1988). In relaxed fibers (pCa 9.2) at a sarcomere length (L_s) of 2.57 $\pm$ 0.04 (mean $\pm$ SD, N = 18), the diameter was 57 $\pm$ 2.3 μm, and L_o was 1.54 $\pm$ 0.27 mm.

Mechanical apparatus and data acquisition

For mechanical measurements, the force transducer was either a model 400A with a 2.2-kHz resonant frequency (Cambridge Technology, Watertown, MA), or a model AE801 with a $\geq$5-kHz resonant frequency (SensoNor, Horton, Norway). Fiber length was controlled with a model 300 servo motor (Cambridge Technology) tuned for a 300-μs step response. Helium-neon laser diffraction was used to measure L_s during experiments (Chase et al., 1993). All signals were recorded digitally with 12-bit resolution at a rate of 0.3–2.0 kHz per channel (Chase et al., 1994). To avoid high frequency aliasing, all signals were low pass filtered ($f_c \leq$ 40% of the sampling rate) with a CyberAmp 380 (Axon Instruments, Foster City, CA) before digitization. Fiber properties could be maintained for long periods of continuous activation by using a protocol first described by Brenner (1983). An example of an activation series for 0.5 mM ATP followed by 5 mM ATP (in the same fiber) can be seen in Fig. 1. For experiments, control measurements (5 mM ATP) made either before or after activations with 5 mM dATP or 0.5 mM ATP gave similar results.

Measurements of k_{tr} (Brenner and Eisenberg, 1986) were as shown in Figs. 4–6, according to the following procedure. First, steady-state isometric force was obtained from the initial portion of each record and was normalized to cross-sectional area by using the measured diameter and assuming circular geometry. In maximum activating solutions (pCa 4.5) the control tension was 260 $\pm$ 48 mN/mm^2 (mean $\pm$ S.D., n = 18), and relaxed force (pCa 9.2) was 1.6 $\pm$ 0.7% of the maximally activated force. To measure k_{tr}, the fiber was then shortened by ~20% L_o, with a 4 $L_o \cdot s^{-1}$ ramp, which reduced force to zero, followed by a rapid (300 μs), underdamped restretch to the initial fiber length. The subsequent force redevelopment kinetics were characterized as previously described (Chase et al., 1994). Briefly, an apparent rate constant was obtained by a linear transformation of the half-time estimate, extrapolating from 50% to 63.2% ($k_{tr} = \tau^{-1} = -\ln 0.5 \cdot (t_{1/2})^{-1}$), and is expressed as reciprocal seconds

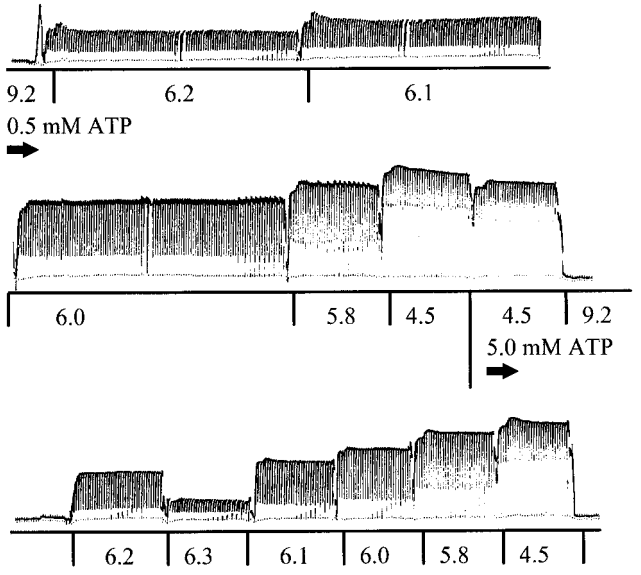


FIGURE 1 Chart record of an activation series comparing contractions with 0.5 mM and 5 mM ATP. Ca^{2+} concentrations (pCa) and solution changes are indicated below the force record. Force transients occur every 5 s because of ramp release/restretch cycles (see Materials and Methods). The top and middle panels show activations with varying pCa and 0.5 mM ATP. At the end of the middle panel the fiber was transferred directly from a pCa 4.5, 0.5 mM ATP solution to a pCa 4.5 solution containing 5 mM ATP for back-to-back comparisons at full activation. After fiber relaxation (pCa 9.2, 5 mM ATP) the control activation series in 5 mM ATP solutions was carried out for comparison.

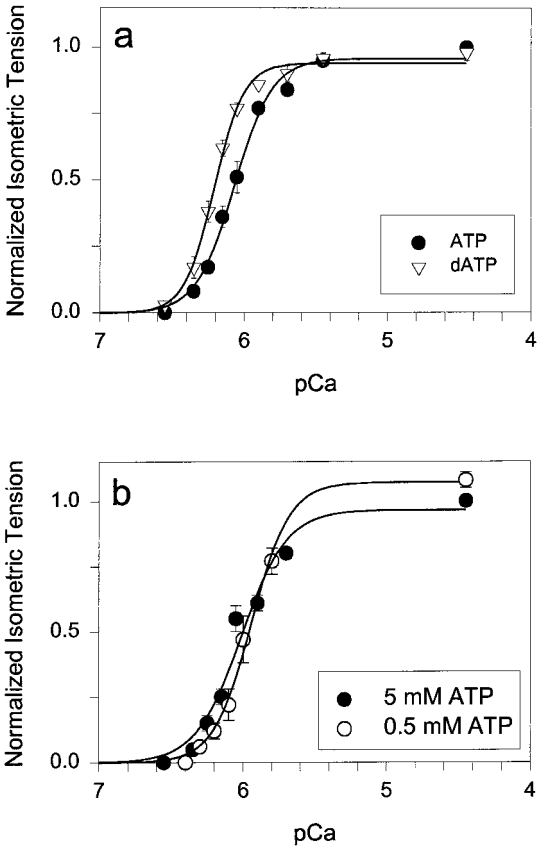


FIGURE 2 Relationship between pCa and steady-state tension. Values are normalized means ± SE for comparison of 5 mM ATP (●) with 5 mM dATP (▽; 10 fibers) or 0.5 mM ATP (○, 9 fibers), and the data were fit with the Hill equation (*solid lines*). In *a* the fit values for half-maximum tension (pK) and slope (*n*) were 6.06 ± 0.02 and 3.3 ± 0.3 (mean ± SE) for 5 mM ATP versus 6.21 ± 0.01 and 4.2 ± 0.3 for 5 mM dATP, with no difference in P_o. In *b* the fit values for 5 mM ATP were 6.00 ± 0.04 (pK) and 2.8 ± 0.5 (*n*) versus 5.95 ± 0.02 (pK) and 3.3 ± 0.3 (*n*) for 0.5 mM ATP, and P_o was increased to 1.08 ± 0.04 ($p < 0.01$).

(s^{-1}). Alternatively, monoexponential fits to the data, obtained with the Simplex method for nonlinear least-squares regression (Caceci and Cacheris, 1984), gave the same results at all submaximum activations tested, but the data were not fit well at maximum activation, resulting in slightly slower k_{tr} values than estimates made from $t_{1/2}$ measurements. Thus all values reported are from $t_{1/2}$ estimates of k_{tr}. For most experiments, k_{tr} was measured without L_s feedback control to minimize the stress placed on fibers during long experimental protocols; this is justified because previous experiments have shown that k_{tr} measured with and without L_s control are linearly correlated, with unclamped measurements underestimating k_{tr} by 20–30% at all levels of Ca^{2+} activation (Chase et al., 1994).

Unloaded shortening velocity (V_u) was measured by the "slack test" method (Edman, 1979). Length steps for slack tests ranged from 5% to 15% of L_o and were applied nonsequentially. Slack time for individual length steps was determined as previously described (Martyn et al., 1994). V_u was determined as the slope of the linear least-squares regression of length steps versus slack time, as demonstrated in Fig. 3, *a* and *b*. Plots of length steps versus slack time were curvilinear at pCa > 6.0 for all substrate conditions, indicating a slowing of V_u as fiber shortening proceeded. This behavior has been reported previously by others for ATP (Brenner, 1986; Martyn et al., 1994; Metzger, 1996; Moss, 1986) and is the subject of a forthcoming manuscript comparing ATP, dATP, and low [ATP]. For the present work we have chosen to report V_u from a single regression fit to the data, which will be more indicative of the slower rate of fiber shortening, especially at low levels of Ca^{2+} activation. To compare the effects 5 mM ATP, 0.5 mM ATP, and 5 mM dATP at varying levels of Ca^{2+} activation levels, measurements of V_u were normalized to the value obtained under control conditions with maximum Ca^{2+} activation (i.e., pCa 4.5, 5 mM ATP).

Fiber solutions

Relaxing and activating solutions were calculated according to the method of Martyn et al. (1994). Solutions were maintained at 0.17 M ionic strength, pH 7.0, and contained (in mM) 15 PCr, 1 P_i, 15 EGTA, at least 40 3-(*N*-morpholino)propanesulfonic acid, 1 free Mg^{2+}, 135 $Na^+ + K^+$, 1 dithiothreitol, 250 units ml^{-1} creatine kinase (CK) (Sigma, St. Louis, MO), dextran T-500 (4% w/v; Pharmacia, Piscataway, NJ), and either 5 ATP, 0.5 ATP, 0.1 ATP, or 5 dATP. The affinity of dATP for Mg^{2+} was assumed to be the same as that of ATP. The Ca^{2+} levels (expressed as pCa = $-\log[Ca^{2+}]$) were established by varying the amount of $Ca(propionate)_2$. Dextran T-500 (4% w/v) was included in all solutions to minimize covariation of myofilament lattice spacing (and fiber diameter) with force (Martyn and Gordon, 1988; Matsubara et al., 1985). All mechanical measurements were made at 10°-12°C.

Modeling of results

The results of steady-state and kinetic tension measurements were used to model cross-bridge and thin filament transition rates by the use of a program developed by Dr. Neil Millar (Millar and Homsher, 1990). This program numerically solves a series of simultaneous differential equations descriptive of the model scheme using the Gear method. The initial conditions before a perturbation allow calculation of steady-state tension from model state populations and, after a perturbation, the rate of tension redevelopment is determined from $t_{1/2}$, as in fibers. Methods for simulating k_{tr} were presented in detail previously (Regnier et al., 1995; Hancock et al., 1997).

RESULTS

Steady-state isometric tension (P), unloaded shortening velocity (V_u), and the rate of tension redevelopment (k_{tr}) were measured during continuous activations in which fiber properties were maintained using a protocol first described by Brenner (1983). An example Ca^{2+} activation series comparing 0.5 mM ATP (low [ATP]) and 5 mM ATP (control) is shown in Fig. 1 and demonstrates that tension levels are stable and reproducible with this protocol. Control measurements for comparison with 5 mM dATP (10 fibers) or low ATP (9 fibers) were made either at the beginning or at the end of experiments with similar results. The effects of dATP and low ATP on the Ca^{2+} sensitivity of P are summarized in Fig. 2. The data are normalized to maximum isometric tension (P_o) under control conditions (i.e., 5 mM ATP, pCa 4.5) and were fitted to the Hill equation (*solid lines*)

$$P = P_o/(1 + 10^{n(pK-pCa)}) \quad (1)$$

where pK is the pCa that gives $0.5P_o$ (midpoint) and n is the Hill coefficient (slope). Changing substrate conditions had only minimal effects on tension. P_o was not affected by dATP (0.99 ± 0.04; mean ± SE) and was elevated only slightly by low [ATP] (1.08 ± 0.04), in agreement with an upcoming report (Regnier et al., 1998). The Ca^{2+} sensitivity of P was increased slightly by dATP, as indicated by a small increase in both pK (0.15 pCa units) and n (from 3.3 ± 0.3 to 4.2 ± 0.3), but neither pK nor n was significantly affected by low [ATP]. These results indicate that the substrate conditions used for these experiments had little effect on the Ca^{2+} sensitivity of steady-state tension in fibers.

Measurements of V_u and k_{tr}

To determine the extent that altering transition rates of the cross-bridge power stroke affects the rate of tension development, we measured V_u and k_{tr} during the same activation. We assumed that for our substrate conditions, V_u correlates with the rate of cross-bridge cycling during isometric tension generation as well as during unloaded fiber shortening (see Introduction). Example measurements of V_u during maximum Ca^{2+} activation (pCa 4.5) are shown in Fig. 3, *a* and *b*, demonstrating that V_u was increased by dATP and decreased by low [ATP]. In agreement with previous reports, V_u at pCa 4.5 averaged 31 ± 4% faster with dATP (Regnier et al., 1998) and 29 ± 7% slower with low [ATP] (Chase and Kushmerick, 1995; Regnier et al., 1998). The effects of dATP and low [ATP] on the Ca^{2+} sensitivity of V_u are summarized in Fig. 3 *c*. The data are normalized to control V_u for each fiber at pCa 4.5 and are plotted versus the value of P (binned in 0.1 unit increments) measured just before length steps. Because control measurements did not differ significantly between fibers used for dATP and low [ATP] experiments, the data were combined for ease of comparison. The normalized relationship between P and V_u was linear (slope = 1.0) for control measurements as the $[Ca^{2+}]$ was varied, in agreement with earlier reports (Far-

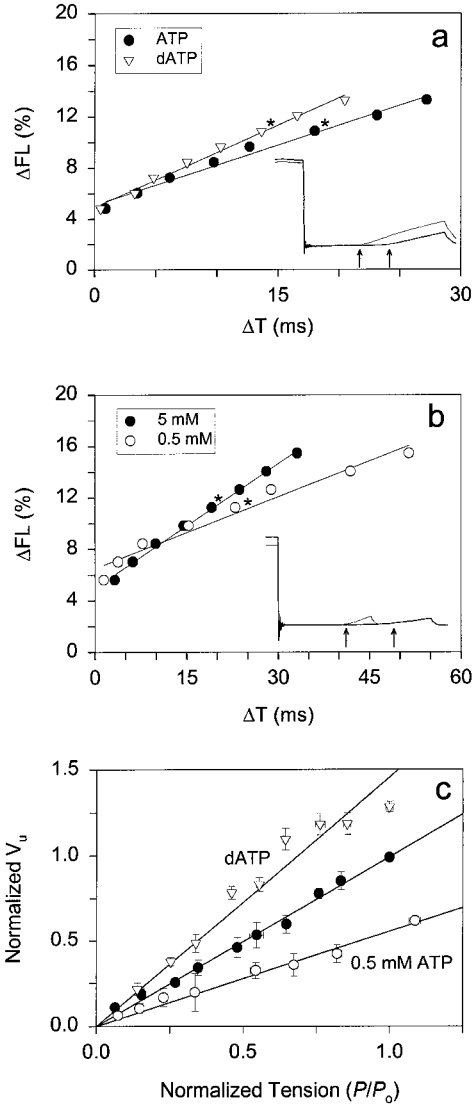


FIGURE 3 Measurement of V_u in fibers. The technique used to determine V_u is demonstrated for a fiber (*a*) comparing 5 mM ATP (●) with 5 mM dATP (▽) and another fiber (*b*) comparing 5 mM ATP (●) with 0.5 mM ATP (○) at pCa 4.5. A series of eight length steps, ranging from 5% to 15% of L_o, were made (nonsequentially) and were plotted versus the measured "slack" times. Individual traces for an 11% length step are shown in the insets, and the "slack" times measured (*arrows*) are indicated by asterisks. The data for each fiber were fitted with a linear regression (*solid lines*; $r^2 = 0.99$), and the slope of the relationship was taken as V_u. For the fiber in *a*, V_u increased from 3.1 fiber lengths per second (FL/s) with 5 mM ATP to 4.3 FL/s with 5 mM dATP. For the fiber in *b*, V_u decreased from 3.1 FL/s with 5 mM ATP to 1.9 FL/s with 0.5 mM ATP. In *c* the V_u measurements for all fibers are summarized and plotted versus tension (P) to compare the effects of changing substrate conditions at different levels of Ca^{2+} activation. Data were binned in 0.1 tension level increments, and the values are normalized (pCa 4.5, 5 mM ATP = 1.0) means ± SE. Data for 5 mM ATP did not differ between fibers used to study dATP or 0.5 mM ATP, so values were grouped for ease of comparison. Solid lines are linear regression fits to the data to demonstrate the elevation of V_u with 5 mM dATP and the depression of V_u with 0.5 mM ATP.

row et al., 1988; Julian, 1971; Julian and Moss, 1981; Julian et al., 1986; Martyn et al., 1994; Metzger and Moss, 1988; Moss, 1986; Wise et al., 1971). V_u was faster with dATP at

all Ca^{2+} activation levels where $P > 0.25P_o$, and the slope of the linear regression increased to 1.46 ± 0.11. In contrast, low [ATP] decreased the slope of the linear regression to 0.56 ± 0.02, and V_u was significantly slower at all Ca^{2+} activation levels where $P > 0.5P_o$. These results indicate that dATP increases whereas 0.5 mM ATP reduces the rate of unloaded fiber shortening at similar levels of submaximum Ca^{2+} activation, suggesting that the rate of cross-bridge cycling may be similarly affected.

The influence of dATP and low [ATP] on k_{tr} is summarized in Figs. 4 and 5. In maximally Ca^{2+} activated fibers, dATP caused a small increase (14 ± 3%) in k_{tr}, and low [ATP] caused a small decrease (11 ± 3%) in k_{tr}, as previously reported (Regnier and Homsher, 1998). The Ca^{2+} sensitivity of k_{tr} was also affected by dATP and low [ATP]. Fig. 4 *a* demonstrates that dATP increases k_{tr} at submaximum as well as at maximum levels of Ca^{2+} activation. The effect of dATP on the Ca^{2+} sensitivity of k_{tr} is summarized in Fig. 4 *c*; it shows that dATP significantly increased k_{tr} at all pCa ≤ 6.2. Fitting the data with Eq. 1 (*solid lines*) was adequate to describe the Ca^{2+}-sensitive portion of the pCa versus k_{tr} relationship, and pK was shifted left by 0.2 pCa units while the slope (*n*) remained unchanged. In comparison, low [ATP] increased k_{tr} slightly at low levels of Ca^{2+} but decreased k_{tr} at higher levels of Ca^{2+}, as demonstrated in Fig. 4 *b*. Although these effects on k_{tr} were relatively small, they were consistent between fibers; the data are summarized in Fig. 4 *d*. Fits with Eq. 1 indicate that pK was not significantly affected by low [ATP], but *n* was reduced by ~50%. In effect, low [ATP] also reduced the Ca^{2+} sensitivity of k_{tr} by half (from a 10-fold to <5-fold range of values) because of the elevation of k_{tr} at low levels of Ca^{2+} coupled with a depression of k_{tr} at high levels of Ca^{2+}.

The leftward shift observed for both the tension versus pCa (Fig. 2 *a*) and k_{tr} versus pCa (Fig. 4 *c*) relationships suggest that increases in k_{tr} with dATP may have resulted solely from an increase in the number of cross-bridges available to participate in the tension-generating process. In contrast, the number of cross-bridges at pCa > 6.0 appears to be either unchanged or slightly reduced by low [ATP] (Fig. 2 *b*), whereas k_{tr} was elevated slightly (Fig. 4 *d*). Therefore, to compare the effects of dATP and low [ATP] at similar Ca^{2+} activation levels, the k_{tr} data in Fig. 4, *c* and *d*, were replotted (binned in $0.1P_o$ tension level increments) as k_{tr} versus *P*. As illustrated in Fig. 5 *a*, k_{tr} was not elevated when similar submaximum tension levels were compared

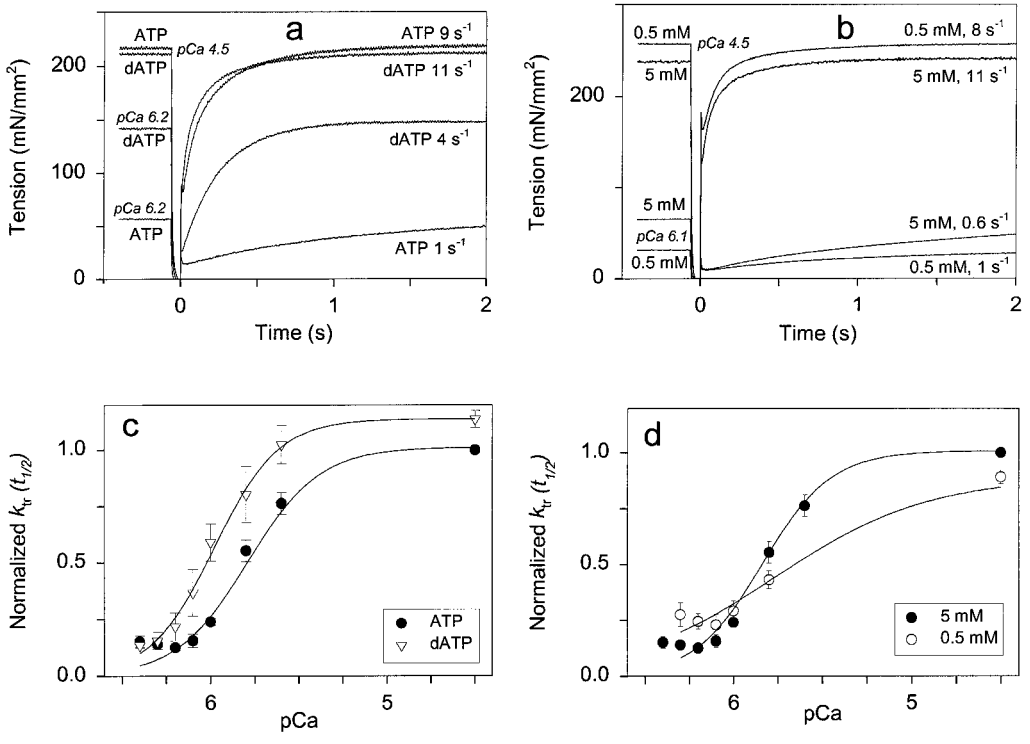


FIGURE 4 The rate of tension redevelopment (k_{tr}) after a release-restretch cycle. (*a*) Example [Ca^{2+}] matched tension traces comparing 5 mM ATP versus 5 mM dATP at pCa 4.5 and pCa 6.2. At pCa 4.5, the steady-state isometric tension before the release-restretch cycle was similar for ATP and dATP, but k_{tr} was increased from 9 s^{-1} to 11 s^{-1} with dATP. At pCa 6.2 isometric tension was increased ~2.6-fold by dATP, and k_{tr} was increased from 1 s^{-1} to 4 s^{-1}. (*b*) Example [Ca^{2+}] matched tension traces comparing 5 mM ATP versus 0.5 mM ATP at pCa 4.5 and 6.2. At pCa 4.5 reduction of the [ATP] from 5 mM to 0.5 mM increased isometric tension slightly (9%) and decreased k_{tr} from 11 s^{-1} to 8 s^{-1}. At pCa 6.2 isometric tension was decreased 40% when the [ATP] was reduced to 0.5 mM, but k_{tr} was increased from 0.6 s^{-1} to 1.0 s^{-1}. The Ca^{2+} sensitivity of k_{tr} is summarized in *c* and *d*, and the data are fitted with Eq. 1 (*solid lines*) for comparison. In *c*, k_{tr} averaged 14 ± 3% faster with dATP, whereas pK was increased from 5.79 ± 0.04 to 5.98 ± 0.02, with no significant change in *n* (2.2 ± 0.3 versus 2.4 ± 0.2 for ATP versus dATP, respectively). In *d*, k_{tr} averaged 11 ± 3% slower with low [ATP], whereas pK was reduced from 6.02 ± 0.02 to 5.87 ± 0.09, and *n* was reduced from 2.4 ± 0.3 to 1.8 ± 0.02.

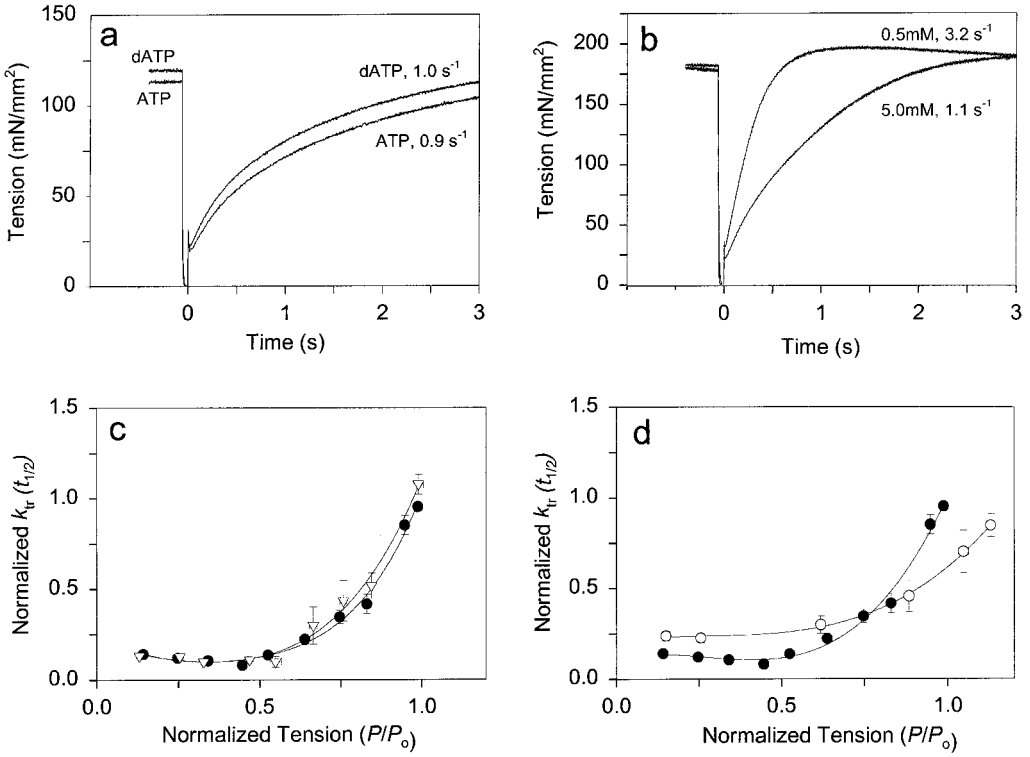


FIGURE 5 Effects of dATP and low [ATP] on the tension versus k_{tr} relationship. (*a*) Example tension-matched k_{tr} traces from the same fiber for ATP and dATP. For the ATP trace, $P = 0.48P_o$ at pCa 6.1, and k_{tr} was 0.9 s^{-1}. For the dATP trace, $P = 0.51P_o$ at pCa 6.2, and k_{tr} was 1.0 s^{-1}. k_{tr} at pCa 4.5, 5 mM ATP was 14 s^{-1}. (*b*) $P = 0.60P_o$ for both 5 mM (pCa 6.0) and 0.5 mM ATP (pCa 5.8). k_{tr} was 1.1 s^{-1} for 5 mM ATP and 3.2 s^{-1} for 0.5 mM ATP. (*c* and *d*) k_{tr} versus tension (*P*) relationships for ATP versus dATP and ATP versus low [ATP], respectively, with k_{tr} values replotted from Fig. 4 after binning the data by tension, rather than pCa. Measurements were binned in 0.1 P_o tension level increments, and values are normalized means ± SE. Data are empirically fit with a nonlinear regression (*solid lines*) and show that k_{tr} is increased only at P_o with dATP, whereas low [ATP] elevates k_{tr} at $P < 0.75\ P_o$ and reduces k_{tr} at greater tension levels.

with dATP. Comparisons over the entire range of tension levels (Fig. 5 *c*) show that k_{tr} was elevated by dATP only during maximum activation, and therefore the increased Ca^{2+} sensitivity of cross-bridge recruitment with dATP (Fig. 2 *a*) can explain the enhanced Ca^{2+} sensitivity of k_{tr} during submaximum activations (Fig. 4 *c*). In contrast, the effects of low [ATP] on k_{tr} persisted in tension-matched traces. As illustrated in Fig. 5 *b*, k_{tr} was faster at low levels of tension with low [ATP], suggesting that the elevation in k_{tr} did not result from an increase in cross-bridge recruitment alone. Comparisons over the entire range of tension levels (Fig. 5 *d*) show that low [ATP] increased k_{tr} at low tension levels and decreased k_{tr} at high tension levels. These results indicate that, unlike the results with dATP, the increase in k_{tr} at low levels of Ca^{2+} with low [ATP] (Fig. 4 *d*) cannot be explained by a concomitant change in cross-bridge recruitment.

DISCUSSION

The experiments described above were designed to test whether the rate of cross-bridge transitions influences tension redevelopment kinetics (k_{tr}) at submaximum as well as maximum levels of Ca^{2+} activation in skinned rabbit psoas fibers. By changing the substrate conditions, we were able to increase (with 5 mM dATP) or decrease (with [ATP] reduced to 0.5 mM) the rate of cross-bridge cycling, as monitored by V_u, and compare measurements of k_{tr} with control conditions (5 mM ATP). The data show that, in maximally Ca^{2+}-activated fibers, changes in V_u correlated with concomitant changes in k_{tr}. These results support the hypothesis that k_{tr} is determined by the properties of myosin in fully activated fibers (Brenner, 1988; Metzger and Moss, 1990). However, at similar submaximum tension levels, increases or decreases in V_u (Fig. 3 *c*) did not result in similar changes in k_{tr} (Fig. 5, *c* and *d*). These data indicate that V_u and k_{tr} have different rate-limiting processes, at least during submaximum activations.

Varied substrate conditions and Ca^{2+} sensitivity of steady state tension

Substrate conditions for our experiments were chosen to have minimal effects on the Ca^{2+} sensitivity of steady-state tension levels while maximizing observable effects on V_u and k_{tr}. As shown in Fig. 2, changes in P_o, pK, and *n* were small, indicating that dATP and moderate lowering of [ATP] had only minor effects on thin filament activation.

Wahr et al. (1997) reported that 5 mM CTP has no significant effect on the tension-pCa relationship and reduced P_o by ~10% in psoas muscle, suggesting that nucleotides that support maximum Ca^{2+}-activated tension in a manner similar to that of ATP do not dramatically alter thin filament activation. In contrast, reducing the [ATP] of activation solutions to lower concentrations (≤0.2 mM) than used in this study (0.5 mM) has been shown to dramatically increase both pK and *n* of the pCa versus tension relationship (Brozovich et al., 1988; Godt, 1974). These effects of substrate conditions on Ca^{2+}-activated tension probably occur because of enhanced thin filament activation via strong cross-bridge attachments. The mechanisms by which the tension-pCa relationship is affected by dATP, in contrast to low [ATP], probably differ, because the time individual cross-bridges occupy strongly bound states is increased when the [ATP] is lowered, but reduced when ATP is replaced with dATP (Regnier and Homsher, 1998). This raises the possibility that the kinetics as well as the number of strongly bound cross-bridges may influence thin filament activation, although the present experiments do not allow us to discern between these two intriguing possibilities.

Varied substrate conditions and cross-bridge cycling

To control for the minor effects of different cross-bridge cycling rates on thin filament activation, data were plotted relative to the isometric tension produced immediately before measurements of V_u (Fig. 3 *c*) and k_{tr} (Fig. 5, *c* and *d*). This method of comparing the data can be used to study the kinetic processes of individual cross-bridges and thin filament regulatory units, assuming that the isometric tension produced at varying Ca^{2+} activation levels is due predominantly to AM·NDP and AM cross-bridges, and these cross-bridge states contribute similarly to tension levels (Dantzig et al., 1991; Ferenczi et al., 1984b; Goldman et al., 1984). When dATP is the substrate for contractions, isometric tension is also thought to be controlled by the level of AM·dADP cross-bridges (Regnier et al., 1998), allowing comparisons of V_u and k_{tr} at similar tension levels. The rate-limiting processes controlling V_u and k_{tr} appear to be the same for ATP and dATP. Fiber shortening velocity is thought to be limited by the rate of cross-bridge dissociation, at least in maximally Ca^{2+}-activated fibers (Ferenczi et al., 1984a; Moss and Haworth, 1984; Siemankowski et al., 1985; Homsher et al., 1997). In contrast, k_{tr} can be affected by several steps at the beginning of the cross-bridge cycle, including, but not limited to, the tension-generating myosin isomerization step (Regnier et al., 1995, 1998; Wahr et al., 1997), as well as by cross-bridge dissociation at low substrate concentrations (Regnier et al., 1998). Although the dissociation of dADP from cross-bridges may be faster, it is probably still the rate-limiting process during fiber shortening with dATP (Regnier et al., 1998). Measurements of the rate of tension relaxation (k_{Pi}), caused by photoliberation of P_i from caged P_i, suggest that the tension-generating step at the beginning of the cross-bridge power stroke is also faster with dATP (Regnier and Homsher, 1998). In addition, dATP binding to and cleavage by myosin are similar to those of ATP, indicating that the concomitant increase in transition rates at the beginning and end of the cross-bridge power stroke allows for the faster V_u with dATP without increasing P_o.

Because the rates that control P_o, V_u, and k_{tr} are the same for ATP and dATP, and cross-bridge dissociation determines P_o and V_u at low [ATP], comparisons should determine if cross-bridge transition rates control the rate of tension development at various levels of Ca^{2+} activation. When dATP replaces ATP, V_u is faster at all activation levels above ~$0.25P_o$, indicating that cross-bridge cycling is also faster. Because the tension-generating step is faster with dATP, k_{tr} might be expected to increase at all submaximum Ca^{2+} activation levels if tension redevelopment kinetics are primarily determined by cross-bridge transition rates. By the same argument, the decrease in V_u with low [ATP] over a similar range in activation levels should correlate with a decrease in k_{tr} at all levels of tension. These relationships are predicted from the two-state model proposed by Brenner and Eisenberg (1986) and Brenner (1988), in which the tension-generating process is directly controlled by [Ca^{2+}] (Fig. 6). Our data do show the predicted increase (dATP) and decrease (low [ATP]) in k_{tr} during maximum Ca^{2+} activation (Figs. 4 and 5). However, contrary to the predictions of the two-state model, k_{tr} is not increased by dATP during submaximum activations and is not decreased, but actually increased slightly, by low [ATP] (Fig. 5, *c* and

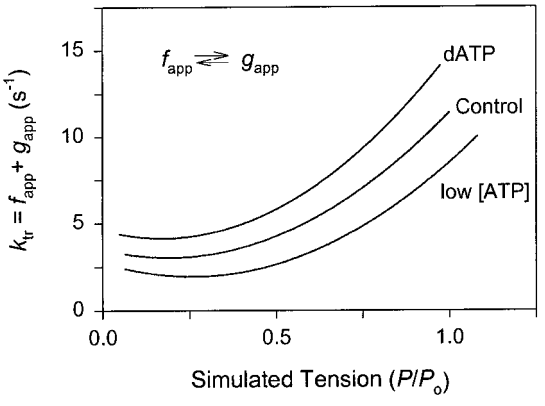


FIGURE 6 Predictions of the effects of dATP and low [ATP] on the k_{tr} versus tension relationship, using the two-state cross-bridge model (Brenner, 1988). In this model Ca^{2+} regulates the amount of tension generated by modulating f_{app} and $k_{tr} = f_{app} + g_{app}$. For simulations of control conditions, values for f_{app} (10 s^{-1}) and g_{app} (3 s^{-1}) were chosen to simulate a maximum rate of ~12 s^{-1} with an ~10-fold increase in k_{tr} as f_{app} is varied, as is seen experimentally. For dATP, values of f_{app} (12 s^{-1}) and g_{app} (4 s^{-1}) were increased to simulate the ~20% increase in k_{tr}, with no change in steady-state tension, which occurs at maximum activation. Lowering the [ATP] was simulated by reducing g_{app} (2 s^{-1}) to account for the 10–20% reduction in k_{tr} and the 8% increase in steady-state tension at maximum activation.

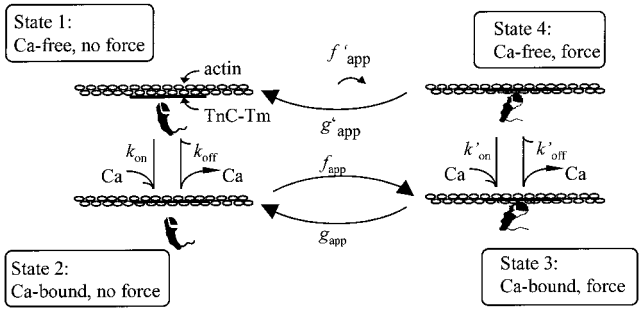
d). This suggests that some process other than cross-bridge transition rates controls k_{tr} during submaximum activations, especially at lower activation levels, where V_u increases with increasing P, but k_{tr} is relatively constant.

Mechanism of control of tension redevelopment kinetics (k_{tr}) by calcium

A likely candidate for the process that controls k_{tr} at submaximum levels of Ca^{2+} is thin filament activation. To explore this possibility, we used a four-state reaction mechanism (Scheme 1), originally proposed by Landesberg and Sideman (1994) and modified by Hancock et al. (1997), to examine how independent manipulation of the rate of cross-bridge cycling or thin filament activation kinetics might influence the k_{tr} versus tension relationship. The features of Scheme 1 have been described in detail elsewhere (Hancock et al., 1997), but the essential features will be reiterated here for the benefit of the reader. Briefly, the processes of Ca^{2+} binding to TnC and tension generation by cross-bridges are modeled as two-state reactions with forward and reverse rate constants. A key feature of Scheme 1 is that no rate constants are dependent on $[Ca^{2+}]$. Instead, Ca^{2+} exerts a regulatory influence on tension via control of the level of thin filament activation and has no direct influence on the kinetic interactions of the cross-bridges. Ca^{2+} binding is modeled as a simple first-order reaction, and for simulation purposes we assumed that $[Ca^{2+}]$ is proportional to the forward rate constant (k_{on}) for thin filament activation (state 1 ↔ state 2). Cross-bridge transition from low tension (state 2) to high tension state(s) (state 3) is governed by the apparent rate constants f_{app} and g_{app}, as in the two-state model (Fig. 6). A unique feature of this model is that Ca^{2+} can come off the thin filament when cross-bridges are in tension-generating states (i.e., state 3 ↔ state 4, governed by k'_{on} and k'_{off}), followed by cross-bridge transition back to low tension states (i.e., state 4 ↔ state 1, governed by g'_{app}; with $f'_{app} < 0.01\ s^{-1}$), thus yielding two groups of tension-generating cross-bridge states (states 3 and 4) and four possible thin filament states. Because it has been demonstrated that k_{tr} is not influenced by cooperative interactions along the thin filament (Chase et al., 1994), this feature has not been included in the model.

Scheme 1 was first employed to model the k_{tr} versus tension relationship for 5 mM ATP (control), 5 mM dATP, and 0.5 mM ATP (Fig. 5, *c* and *d*, shown above). For simulations of the control k_{tr} versus tension relationship, conditions were chosen that best simulated the data for 5 mM ATP in Fig. 5 *c*. Values assigned to k_{on} ($30\ s^{-1}$) and k_{off} ($5\ s^{-1}$) at maximum Ca^{2+} activation were taken from earlier work (Regnier et al., 1995, 1998), and the apparent rate constants for cross-bridge cycling (f_{app}, g_{app}, and g'_{app}) were set to allow for a simulated maximum k_{tr} of $12\ s^{-1}$, a minimum k_{tr} of $1\ s^{-1}$ (determined by g_{app} and g'_{app}), and a calculated ATPase of $\sim 1\ s^{-1}$ at full activation, in addition to providing a linear relationship between tension and ATPase as the simulated Ca^{2+} activation level was varied. As in the two-state model (Fig. 6), g_{app} limits how slow k_{tr} can become at low levels of activation, whereas the sum of $f_{app} + g_{app}$ determines the maximum possible k_{tr}. Unlike in the two-state model, however, smaller ratios of k_{on}:k_{off} can limit maximum k_{tr}. It was found that higher f_{app}:g_{app} ratios (>10:1) best simulated the independence of k_{tr} on Ca^{2+} activation at low levels of tension ($p < 0.5P_o$), while allowing the 10-fold increase in k_{tr} that occurs in fibers at high levels of tension. Increasing g_{app} independently of g'_{app} introduced an undesired curvilinearity into the tension-ATPase relationship, even though it reduced the cross-bridge duty cycle and maintained the characteristic shape of the tension versus k_{tr} relationship. Furthermore, varying k'_{on} and k'_{off} independently from k_{on} and k_{off} (respectively) had little effect on simulations. Therefore, for simplicity, k'_{on}, k'_{off}, and g'_{app} were covaried with k_{on}, k_{off}, and g_{app}, respectively, to model the various experimental conditions.

For simulations of dATP, f_{app}, g_{app}, and g'_{app} were increased to account for faster cross-bridge cycling and the results of Regnier et al. (1998) and Regnier and Homsher (1998), and to simulate low [ATP], g_{app} and g'_{app} were reduced. The k_{tr} versus tension relationships generated from simulations are shown in Fig. 7, and the rate constants used are summarized in Table 1. These adjustments of cross-bridge apparent rate constants were sufficient to character-



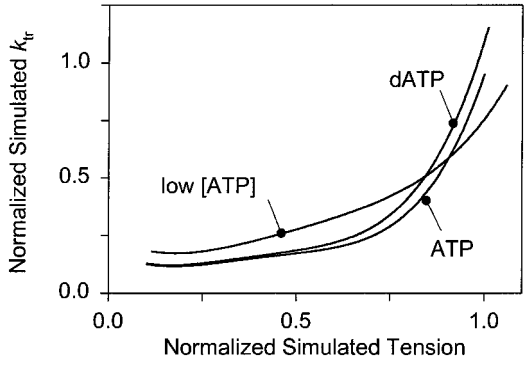


FIGURE 7 Model simulations of the k_{tr} versus tension relationship for ATP, dATP, and low [ATP]. Details of the model are provided in the text of the Discussion, and the values used for simulations are given in Table 1. Values for k_{tr} were determined from $t_{1/2}$ of the simulated tension transients, as in experimental measurements (see Materials and Methods), and are normalized to maximum simulated tension and k_{tr} ($12\ s^{-1}$) under control conditions.

TABLE 1 Rate constants used for simulation data in Fig. 8, using Scheme 1

Condition	k_{on} (s^{-1})	k_{off} (s^{-1})	f_{app} (s^{-1})	g_{app} (s^{-1})
Control	30	5	13	1
dATP	30	5	17	1.1
Low [ATP]	30	1	11	0.7
CDZ	30	2	13	1
aTnC	30	0.1	13	1

Values for k'_{on}, k'_{off}, and g'_{app} are the same as for k_{on}, k_{off}, and g_{app}, respectively. k_{on} was set at 30 s^{-1} for simulations of maximum Ca^{2+} activation (pCa 4.5).

ize the effect of dATP on the k_{tr} versus tension curve (compare Fig. 7 with Fig. 5 *c*), such that simulated k_{tr} values were elevated only at high levels of tension. Furthermore, the simulations predict a faster dATPase activity compared to ATPase activity (Regnier et al., 1998), as well as the small increase in Ca^{2+} sensitivity of steady-state tension shown in Fig. 2. In contrast to dATP, simulations of low [ATP] by reduction of g_{app} and g'_{app} alone were not sufficient to model the k_{tr} versus tension relationship seen during experiments comparing 0.5 mM ATP and 5 mM ATP. Reduction of g_{app} and/or g'_{app} resulted in slower simulated k_{tr} values at all tension levels, whereas k_{tr} was reduced only at high levels of tension in experiments and was elevated at low levels of tension (Fig. 5 *d*). To simulate an elevated k_{tr} at low levels of tension, it was necessary to also decrease k_{off} alone or k_{off} and k'_{off} together. This adjustment allowed the simulations of low [ATP] to characterize the k_{tr} versus tension relationship for 0.5 mM ATP in fibers (compare Fig. 7 *a* with Fig. 5 *d*). The implications of this are discussed below. The simulations also predict a reduced ATPase rate and, interestingly, a leftward shift of the tension-pCa relationship. Although this did not occur in our experiments, even lower concentrations of ATP (< 0.2 mM) have been shown to shift the pCa versus tension relationship leftward (Brozovich et al., 1988; Godt, 1974).

Scheme 1 is a useful tool for describing the relative contribution of thin filament activation kinetics versus the kinetics of cross-bridge cycling in determining the rate of tension generation as the level of Ca^{2+} activation is varied. However, we recognize that it presents an oversimplified picture of both thin filament activation processes and the actomyosin interactions that occur during tension generation. As such, it cannot describe a variety of data in the literature. For example, explanation of P_i transients (k_{Pi}), pressure transients, and the kinetic differences between these transients and k_{tr} requires a more sophisticated, strain-dependent cross-bridge model (Regnier et al., 1995; Homsher et al., 1997). Despite these limitations, Scheme 1 can simulate the relationship between steady-state tension and the rate of tension development in skinned muscle fibers. Furthermore, it demonstrates that the curvilinearity of the k_{tr} versus tension relationship is probably due to Ca^{2+} limiting a process preceding the cross-bridge force-generating step at low levels of Ca^{2+} activation in skinned psoas fibers. Finally, it provides an explanation of why the rate of cross-bridge cycling was correlated with k_{tr} only at high levels of Ca^{2+}-activated tension when ATP was replaced with dATP or the [ATP] was reduced from 5 mM to 0.5 mM.

The idea that Ca^{2+} controls the rate of tension development by some process before the beginning of the cross-bridge power stroke is supported by several lines of evidence. As steady-state tension (P) is varied with [Ca^{2+}], k_{tr} increases ~10-fold, whereas k_{Pi}, which is significantly faster than k_{tr}, is either unaffected or only slightly reduced at low levels of Ca^{2+} (Araujo and Walker, 1996; Dantzig et al., 1992; Millar and Homsher, 1990; Walker et al., 1992). These differential Ca^{2+} sensitivities indicate that the tension-generating step is not directly controlled by Ca^{2+}, in agreement with the interpretation of pressure jump experiments at varying levels of Ca^{2+} activation (Fortune et al., 1994). This conclusion is bolstered by observations that when the tension-generating mechanism is altered independently of [Ca^{2+}], k_{tr} and k_{Pi} covary. For example, when P is reduced by increasing the [P_i] of activation solutions, both k_{tr} and k_{Pi} are increased, whereas both are decreased by inhibition of P with butanedione monoxime (BDM). Taken together, these results were best interpreted by using a model in which Ca^{2+} regulates a process before a BDM-sensitive tension-generating cross-bridge step (Regnier et al., 1995). That Ca^{2+} controls a process preceding tension generation is also compatible with the results of McKillop and Geeves (1993) and Geeves and Conibear (1995), who proposed a three-state model for thin filament activation and strong cross-bridge binding. In this model Ca^{2+} regulates cross-bridge transition from a blocked state (B) to a closed state (A) where the cross-bridge is relatively weakly bound. Transition from A to an open state (R) allows strong cross-bridge binding, and this transition is much less Ca^{2+} sensitive, but is greatly affected by BDM.

There is a growing amount of evidence to suggest that the kinetics of thin filament activation is the process that limits k_{tr} during submaximum Ca^{2+} activations. Chase et al. (1994) found that when native TnC was extracted and replaced with an activated form of TnC (aTnC), k_{tr} was at maximum and was insensitive to [Ca^{2+}], even when submaximum tension levels were achieved in the absence of Ca^{2+} by reconstitution with a mixture of aTnC and cardiac TnC (Fig. 8 *a*). They concluded that k_{tr} reflects the dynamics of activation for individual thin filament regulatory units and that the Ca^{2+} modulation of k_{tr} is affected primarily by Ca^{2+} binding to TnC. Similarly, when thin filaments were continuously activated by low concentrations of NEM-S1, k_{tr} was nearly at maximum and was insensitive to Ca^{2+} in skinned rabbit psoas fibers (Swartz and Moss, 1992). Furthermore, the influence of thin filament activation kinetics on k_{tr} was demonstrated more directly by Regnier et al. (1996), who found that the Ca^{2+}-sensitizing agent calmidazolium (CDZ) increases k_{tr} at submaximum but not at maximum levels of Ca^{2+} activation (Fig. 8 *a*). CDZ increases the activated time of individual thin filament regulatory

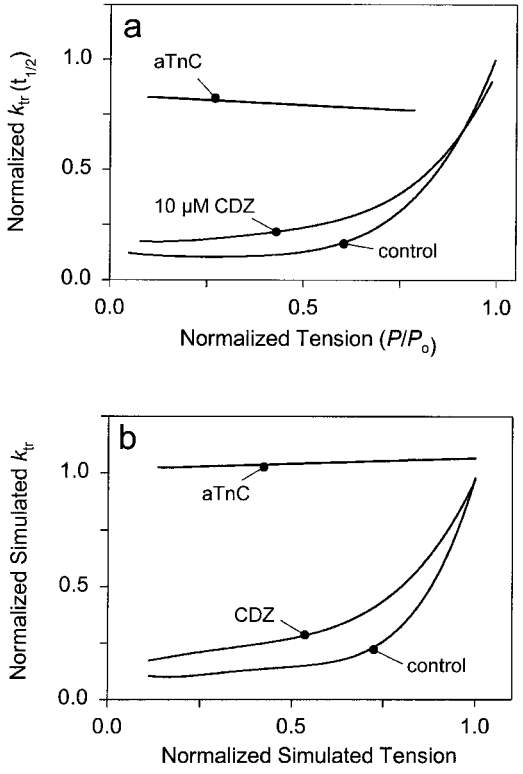


FIGURE 8 Comparison of the effect of calmidazolium (CDZ) or activating TnC (aTnC) on the k_{tr} versus tension relationship. (*a*) Data replotted from Regnier et al. (1996) (CDZ) and Chase et al. (1994) (aTnC), demonstrating that altering the Ca^{2+} binding kinetics of TnC can exert a large influence on k_{tr} at submaximum levels of Ca^{2+}-activated tension. (*b*) Simulations of the data in *a*, using Scheme 1 and reduction of k_{off} and k'_{off}. Values are normalized to maximum experimental (*a*) or simulated (*b*) tension, and k_{tr} (12 s^{-1}) under control conditions and simulation rate constants used to generate the curves in *b* are given in Table 1.

units by decreasing the rate of Ca^{2+} release from TnC (El-Saleh and Solaro, 1987; Johnson et al., 1994; Wahr et al., 1993) but, at the concentrations used, has no effect on P_o, acto-HMM ATPase, or in vitro F-actin motility (Regnier et al., 1996). The results of these two studies demonstrate that manipulation of thin filament activation kinetics has a strong effect on k_{tr} in submaximally activated fibers.

To determine if Scheme 1 predicts the effects of CDZ and aTnC on the k_{tr} versus tension relationship, k_{off} and k'_{off} were reduced to simulate a slower Ca^{2+} dissociation from TnC. The values that best fit the experimental data (Fig. 8 *a*) are shown in Table 1, and the results of simulations are shown in Fig. 8 *b*. Comparisons of the experimental and simulated k_{tr} versus tension support the hypothesis that elevation of k_{tr} at low levels of tension is due to slower Ca^{2+} dissociation from TnC with CDZ, whereas aTnC is in a continually activated form, regardless of [Ca^{2+}] in activation solutions. Simulations using Scheme 1 suggest that elevation of k_{tr} at low levels of Ca^{2+}-activated tension could also result from slowed thin filament activation kinetics, as indicated by the need to decrease k_{off} and k'_{off} to simulate the results of low [ATP] experiments (see Figs. 5 *d* and 7). Although the mechanism by which this would occur is unknown, it is possible that increasing the time that cross-bridges spend in strongly bound states inhibits thin filament deactivation via steric inhibition of tropomyosin movement or by altering the Ca^{2+} binding kinetics of TnC. Regardless of the mechanism by which this occurs, independent manipulation of cross-bridge cycling rates (with dATP or low [ATP]) or thin filament activation kinetics (with CDZ or aTnC) demonstrates that k_{tr} is controlled by a process preceding tension generation at submaximum levels of Ca^{2+} activation. Because these data can be well described by simulations with Scheme 1, we conclude that k_{tr} is regulated by the kinetics of the thin filament in submaximally activated fibers, whereas cross-bridge transition rates determine k_{tr} in maximally activated fibers.

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