Cooperative Mechanisms in the Activation Dependence of the Rate of Force Development in Rabbit Skinned Skeletal Muscle Fibers

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ABSTRACT Regulation of contraction in skeletal muscle is a highly cooperative process involving Ca^{2+} binding to troponin C (TnC) and strong binding of myosin cross-bridges to actin. To further investigate the role(s) of cooperation in activating the kinetics of cross-bridge cycling, we measured the Ca^{2+} dependence of the rate constant of force redevelopment (k_{tr}) in skinned single fibers in which cross-bridge and Ca^{2+} binding were also perturbed. Ca^{2+} sensitivity of tension, the steepness of the force-pCa relationship, and Ca^{2+} dependence of k_{tr} were measured in skinned fibers that were (1) treated with NEM-S1, a strong-binding, non–force-generating derivative of myosin subfragment 1, to promote cooperative strong binding of endogenous cross-bridges to actin; (2) subjected to partial extraction of TnC to disrupt the spread of activation along the thin filament; or (3) both, partial extraction of TnC and treatment with NEM-S1. The steepness of the force-pCa relationship was consistently reduced by treatment with NEM-S1, by partial extraction of TnC, or by a combination of TnC extraction and NEM-S1, indicating a decrease in the apparent cooperativity of activation. Partial extraction of TnC or NEM-S1 treatment accelerated the rate of force redevelopment at each submaximal force, but had no effect on kinetics of force development in maximally activated preparations. At low levels of Ca^{2+}, 3 μM NEM-S1 increased k_{tr} to maximal values, and higher concentrations of NEM-S1 (6 or 10 μM) increased k_{tr} to greater than maximal values. NEM-S1 also accelerated k_{tr} at intermediate levels of activation, but to values that were submaximal. However, the combination of partial TnC extraction and 6 μM NEM-S1 increased k_{tr} to virtually identical supramaximal values at all levels of activation, thus, completely eliminating the activation dependence of k_{tr}. These results show that k_{tr} is not maximal in control fibers, even at saturating $[Ca^{2+}]$, and suggest that activation dependence of k_{tr} is due to the combined activating effects of Ca^{2+} binding to TnC and cross-bridge binding to actin.

KEY WORDS: cooperativity • regulation of contraction • skeletal muscle • myosin

INTRODUCTION

In skeletal muscle, Ca^{2+} binding to low affinity sites on troponin C (TnC)[1] initiates a series of molecular events that ultimately allow strong binding of cross-bridges to actin and subsequent force development (for review see Gordon et al., 2000). During relaxation, dissociation of Ca^{2+} from TnC initiates inactivation of the thin filament and subsequent detachment of cross-bridges from actin. While Ca^{2+} binding to TnC is required for contraction, complete activation of tension and the kinetics of tension development appears to arise from interactive effects of Ca^{2+} binding to TnC and cross-bridge binding to actin (Lehrer, 1994; Gordon et al., 2000). Previously, McKillop and Geeves (1993) proposed a general model for the regulation of muscle contraction in which the thin filament exists in three distinct states; i.e., blocked, closed, and open. In this model, Ca^{2+} binding to TnC shifts the thin filament from the blocked state to the closed state, which appears to involve Ca^{2+}-dependent movement of tropomyosin (Haselgrove, 1973; Huxley, 1973; Parry and Squire, 1973). In the presence of Ca^{2+}, strong binding of cross-bridges to actin shifts the thin filament from the closed state to the open state. Thus, complete activation of the thin filament in terms of numbers of strongly bound cross-bridges requires both Ca^{2+} and strong-binding myosin cross-bridges, an idea which has gained considerable experimental support (Gordon et al., 2000).

It is well established that the regulation of Ca^{2+}-activated force in striated muscles involves cooperative interactions within the thin filament. This is evident, for example, in the biphasic form of the force-pCa relationship, which is steeper at low levels of Ca^{2+} than at high because of greater intermolecular cooperation at forces less than ∼0.50 P_o (for review see Moss, 1992). Furthermore, most investigators have observed that the steepness of the force-pCa relationship is relatively high in skeletal muscle, i.e., Hill coefficients of 4 and greater (Moss, 1992). Since skeletal TnC has just two low affinity Ca^{2+} binding sites (for review see Grabarek et al., 1992), the large Hill coefficients observed in skinned fi-

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[1]*Abbreviations used in this paper:* NEM, *N*-ethylmaleimide; NEM-S1, NEM-modified myosin subfragment-1; TnC, troponin C.

bers are generally thought to manifest cooperative processes in the activation of contraction. For example, activation of one region of the thin filament by Ca^{2+} or strong-binding cross-bridges appears to increase the likelihood of activation of adjacent regions (for review see Lehrer, 1994).

A model of activation proposed by Campbell (1997) predicts that cooperative interactions within the thin filament are a dynamic component of force development in striated muscles. Cross-bridge binding to the thin filament was proposed to cooperatively increase the number of cross-bridges bound to the thin filament and the rate of subsequent cross-bridge binding. In fact, there are several reports that the rate of force redevelopment (k_{tr}) in skeletal muscles varies as much as 10-fold when [Ca^{2+}] is varied from threshold to maximal values (Brenner and Eisenberg, 1986; Metzger et al., 1989; Swartz and Moss, 1992; Palmer and Kentish, 1998). Typically, k_{tr} is thought to be the sum of forward and reverse rate constants describing the transition between non–force- and force-generating states, i.e., f_{app} and g_{app}, respectively (Brenner and Eisenberg, 1986). A model in which f_{app} varies with the level of activator Ca^{2+} has been proposed to account for the activation dependence of k_{tr} (Brenner, 1988), but this and other models (Landesberg and Sideman, 1994; Hancock et al., 1997; Regnier et al., 1998; Brenner and Chalovich, 1999) do not account for the effects of strong-binding cross-bridges on k_{tr} (Swartz and Moss, 1992). According to Campbell's model, the Ca^{2+} dependence of k_{tr} is not due to direct effects on either f_{app} or g_{app}, but instead arises because of effects of strongly bound cross-bridges to cooperatively recruit noncycling cross-bridges. At low [Ca^{2+}], the number of noncycling cross-bridges is high, so that progressive cooperative recruitment of cross-bridges from this pool would slow the overall rate of force development. The first cross-bridges that bind recruit additional cross-bridges, which then bind and recruit still more cross-bridges, and so forth, until the force becomes steady. At higher levels of Ca^{2+}, cooperative slowing of the rate of force redevelopment would be minimized because Ca^{2+} binding to TnC would immediately recruit most cross-bridges into the cycling pool, leaving few cross-bridges available for cooperative recruitment. If this model is correct, eliminating the effects of molecular cooperation would be expected to eliminate the activation dependence of k_{tr}, i.e., k_{tr} should be similar at all levels of activation. Previous studies on skinned fibers used a strong-binding, non–force-generating derivative of myosin subfragment-1 (i.e., N-ethylmaleimide–modified myosin subfragment-1 or NEM-S1) to increase the number of strongly bound cross-bridges and found that NEM-S1 increased k_{tr} at submaximal levels of activation, i.e., at low [Ca^{2+}] or low force (Swartz and Moss, 1992). However, activation dependence of k_{tr} was not completely eliminated by NEM-S1, since k_{tr} at intermediate levels of activation was still less than at low or high levels of activation.

The present study was done to further investigate the mechanisms underlying the activation dependence of the rate of force development in skinned skeletal muscle fibers, which was done by varying the degree of molecular cooperation during activation. Fibers were treated with NEM-S1 to promote strong binding of endogenous cross-bridges (Swartz and Moss, 1992), or subjected to partial extraction of TnC to limit the spread of activation along the thin filament (Brandt et al., 1984, 1990; Moss et al., 1985), or both. Our findings show that it is necessary to apply both NEM-S1 and the partially extracted TnC to completely eliminate the activation dependence of k_{tr}.

MATERIALS AND METHODS

Experimental Solutions

The composition of relaxing solution was as follows (in mM): 100 KCl, 20 imidazole, 4 MgATP, 2 EGTA, and 1 free Mg^{2+}, pH 7.0 at 22°C. Activating solution contained (in mM): 79.2 KCl, 20 imidazole, 14.5 creatine phosphate, 7 EGTA, 5.42 $MgCl_2$, and 4.68 ATP, with [Ca^{2+}]$_{free}$ ranging from 1 nM (i.e., 9.0 pCa) to 32 μM (i.e., 4.5 pCa), pH 7.0 at 15°C and an ionic strength of 180 mM. A computer program (Fabiato, 1988) was used to calculate the final concentrations of each metal, ligand, and metal–ligand complex based on stability constants listed by Godt and Lindley (1982).

Skinned Fiber Preparations

Fast-twitch skeletal muscle fibers were obtained from the psoas muscles of adult New Zealand rabbits. Bundles of ∼30 fibers were dissected from psoas muscles while in relaxing solution. Each bundle was tied with 4/0 suture to glass capillary tubes, placed in relaxing solution containing 1% Triton X-100 for 4–5 h at 4°C, and then stored in relaxing solution containing 50% glycerol at −20°C for up to 3 wk. Chemically skinned fibers were ready for experimental use 1–2 d after dissection.

Experimental Apparatus and General Protocols

Before each experiment, an individual skinned psoas fiber was carefully pulled from one end of a fiber bundle and mounted between a force transducer (model 400A; Aurora Scientific) and a DC torque motor (model 308B; Aurora Scientific) in an experimental apparatus similar to one described previously (Moss, 1979). A fiber segment 1.0–1.5 mm long remained exposed to the solution between the force transducer and the motor. Before mechanical measurements, the experimental apparatus was set on the stage of an inverted microscope (Carl Zeiss, Inc.). The length and force signals of each fiber were digitized at 1 kHz, using a 12-bit A/D converter (model AT-MIO-16F-5; National Instruments Corp.), and displayed and stored on a personal computer using customized software (LabView Full Development System for Windows, version 5.01; National Instruments Corp.). Length changes were driven by computer-generated voltage commands, which were output to the torque motor via a 12-bit D/A converter. All experiments were performed at 15°C and at a sarcomere length of ∼2.35 μm in relaxing solution. During activation and relaxation, sarcomere length and fiber dimensions were recorded on videotape using a video camera (model WV-BL730; Panasonic) and VHS recorder (model SVO-1420; Sony).

Mechanical measurements were first performed on all fibers under control conditions (N = 33). Next, fibers were either subjected to (1) partial extraction of TnC (N = 15), (2) treatment with NEM-S1 (N = 18), or (3) both (N = 15); and mechanical measurements were repeated.

Experimental Treatments

Partial Extraction of Troponin C. Approximately 50% of endogenous TnC was specifically extracted from thin filaments of psoas fibers using a modification of a method reported previously (Metzger and Moss, 1991). After initial measurements of mechanical properties, each psoas fiber was bathed for 1–3 min in a TnC extraction solution containing 10 mM imidazole, 5 mM EGTA and 200 μM trifluoperazine dihydrochloride (TFP; Sigma-Aldrich), pH 7.0 at 15°C. The fiber was subsequently washed three to four times in fresh relaxing solution to remove any residual TFP. Sarcomere length during the extraction protocol was ∼2.35 μm. Maximal Ca^{2+}-activated force was significantly reduced after partial extraction of TnC, ranging from 0.15 to 0.65 of the P_o measured in the preextracted fiber. Measurements of k_{tr} and force were subsequently obtained as functions of pCa in the partially TnC-extracted fibers. Control experiments demonstrated that incubating the partially TnC-extracted fibers for ∼30 s in relaxing solution containing 0.6 mg skeletal TnC/ml resulted in complete recovery of steady-state force and restored Hill coefficients to control values (data not shown).

Preparation and Use of NEM-S1. Myosin subfragment 1 (S1) was purified from rabbit fast-twitch skeletal muscle and modified with *N*-ethylmaleimide (NEM) as described previously (Swartz and Moss, 1992). Although NEM-S1 significantly increases myo-

sin ATPase activity in solutions containing myosin, regulated actin, and Ca^{2+} (Williams et al., 1984; Greene et al., 1987), it exhibits no ATPase activity of its own (Williams et al., 1984). NEM-S1 forms long lasting complexes with actin in the presence or absence of Ca^{2+} and ATP (Swartz and Moss, 1992). In the present study, the concentration of NEM-S1 was estimated by absorbance at 280 nm (with light-scattering correction performed at 320 nm) using a mass absorptivity of 0.75 and a molecular mass of 118,000 D for S1. A stock solution of NEM-S1 was prepared by overnight dialysis against a solution containing 20 mM imidazole, pH 7.0, and 1 mM DTT. A working solution of NEM-S1 was prepared immediately before use by mixing equal volumes of NEM-S1 stock and a 2× stock of pCa 9.0 solution. NEM-S1 was adjusted to the desired concentration by adding the appropriate amount of 1× pCa 9.0 solution. Before mechanical measurements, each fiber was incubated for 15 min at 15°C in solution of pCa 9.0 containing either 3, 6, or 10 μM NEM-S1. The skinned fiber was subsequently transferred to activating solutions of varying pCa without NEM-S1 to measure the steady-state force and the rate of force redevelopment. After mechanical measurements, the skinned fiber was returned to the pCa 9.0 solution containing NEM-S1.

Specific Experimental Protocols

Rate of Tension Redevelopment. The rate constant of force redevelopment (k_{tr}) in skinned psoas fibers was assessed using a modification of an experimental protocol described previously (Brenner and Eisenberg, 1986). Measurement of k_{tr} involves a mechanical slack-restretch maneuver to dissociate bound cross-bridges from actin in a steadily Ca^{2+}-activated preparation. Each skinned fiber was transferred from relaxing to activating solutions of varying pCa (i.e., pCa 6.6–4.5) and allowed to generate steady-state force. The preparation was rapidly (<2 ms) slackened by 20% of its original length, resulting in an abrupt reduction of force to zero (i.e., <5% of steady isometric force). This was followed by a brief period of unloaded shortening (e.g., 20–25 ms) after which the fiber was rapidly (<2 ms) restretched to its initial length. Force redevelopment following the slack-restretch maneuver and force recovery to the original steady-state value reflect the rate of cross-bridge cycling between weakly bound and strongly bound, force-generating states (Brenner and Eisenberg, 1986). A k_{tr}-pCa relationship was obtained by first maximally activating the skinned fiber in a solution of pCa 4.5, and then in a series of submaximally activating solutions between pCa 6.6 and 5.6. To assess any decline in maximal k_{tr}, the fiber was activated in a solution of pCa 4.5 at the end of each protocol. The reference value of max-
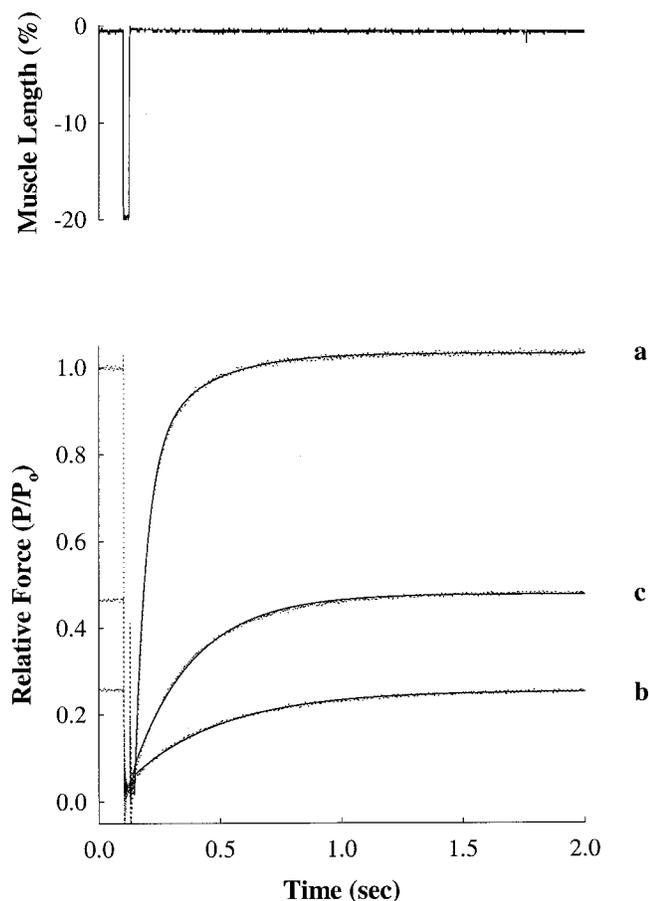

FIGURE 1. Force redevelopment traces in a skinned psoas fiber at maximal and submaximal levels of Ca^{2+} activation. (Top) Depicts the mechanical release-restretch maneuver imposed on a skinned psoas fiber to determine the rate constant of force redevelopment (k_{tr}). The length change was sufficient to slacken the fiber, so that shortening velocity was maximal. (Bottom) Changes in submaximal force in solutions of pCa 6.1 (trace b) and pCa 6.0 (trace c) in a skinned psoas fiber under control conditions. The change in force is expressed relative to the maximum force (P_o) generated by the same fiber under control conditions when exposed to a solution of pCa 4.5 (trace a). The solid lines are exponential fits to the data: single exponentials were adequate for fits at low and intermediate [Ca^{2+}], whereas two exponentials were required for fits at maximal [Ca^{2+}]. These fits are for illustrative purposes only, as k_{tr} was calculated in each case from the half-time of tension rise, as described in materials and methods. Table IV summarizes the double exponential fits at low, intermediate, and high levels of activation.

TABLE I

Steady-state Mechanical Measurements in Skinned Single Psoas Fibers after Partial Tnc Extraction and Treatment with NEM-S1

Group	P_o	$P_{pCa9.0}$	n_2	n_1	pCa_{50}	ΔpCa_{50}
	mN/mm^2	mN/mm^2				
Control (15)	159.7 ± 8.0	0.96 ± 0.13	7.25 ± 0.44	2.73 ± 0.09	5.97 ± 0.01	
$(-)$ TnC (15)	$65.3 \pm 3.7^{\parallel}$	0.78 ± 0.09	$4.53 \pm 0.24^{\parallel}$	2.31 ± 0.11	$5.75 \pm 0.02^{\parallel}$	$0.22 \pm 0.03*$
$(-)$ TnC/3 μM NEM-S1 (7)	$54.8 \pm 4.5^{\parallel}$	$1.24 \pm 0.20^{\parallel\P}$	$3.21 \pm 0.16^{\parallel\P}$	2.33 ± 0.12	$5.76 \pm 0.02^{\parallel}$	$0.01 \pm 0.01^{\ddagger}$
$(-)$ TnC/6 μM NEM-S1 (8)	$55.6 \pm 3.5^{\parallel}$	$5.86 \pm 0.50^{\parallel\P}$	$1.71 \pm 0.11^{\parallel\P}$	2.14 ± 0.21	$5.89 \pm 0.02^{\parallel\P}$	$0.15 \pm 0.01^{\ddagger}$
Control (18)	165.1 ± 6.4	0.74 ± 0.11	7.94 ± 0.44	2.76 ± 0.08	6.00 ± 0.01	
3 μM NEM-S1 (7)	164.5 ± 10.7	$2.42 \pm 0.32^{\parallel}$	$4.63 \pm 0.22^{\parallel}$	2.50 ± 0.09	6.04 ± 0.02	$0.03 \pm 0.01^{\S}$
6 μM NEM-S1 (8)	$149.2 \pm 3.9^{\parallel}$	$8.78 \pm 1.04^{\parallel}$	$3.14 \pm 0.19^{\parallel}$	2.42 ± 0.10	$6.06 \pm 0.02^{\parallel}$	$0.07 \pm 0.01^{\S}$
10 μM NEM-S1 (3)	$108.8 \pm 2.8^{\parallel}$	$12.45 \pm 1.01^{\parallel}$	$1.77 \pm 0.04^{\parallel}$	2.14 ± 0.26	$6.12 \pm 0.04^{\parallel}$	$0.12 \pm 0.03^{\S}$

All values are expressed as means $\pm$ SEM, with the number of skeletal fibers given in parentheses. $(-)$ TnC, partial TnC extraction; P_o, maximal Ca^{2+}-activated tension measured in pCa 4.5; $P_{pCa9.0}$, Ca^{2+}-independent resting tension measured in oCa 9.0; n_2, Hill coefficient for Ca^{2+}-activated force <0.50 P_o; n_1, Hill coefficient for Ca^{2+}-activated force >0.50 P_o; pCa_{50}, pCa required for half-maximal activation.

$^{\parallel}$Significantly different from the control.

¶Significantly different from TnC-extracted fibers.

$*\Delta pCa_{50}$ $(-)$ TnC versus Control.

$^{\ddagger}\Delta pCa_{50}$ $(-)$ TnC/NEM-S1 versus $(-)$ TnC.

$^{\S}\Delta pCa_{50}$ NEM-S1 versus Control.

imal k_{tr} for each submaximal activation was obtained by interpolation between the initial and final measurements of maximal k_{tr}. The apparent rate constants of force redevelopment (k_{tr}) were estimated by linear transformation of the half-time of force redevelopment, i.e., $k_{tr} = 0.693/t^{1/2}$, as described previously (Regnier et al., 1998). Most k_{tr} measurements were done without sarcomere length control, although experiments performed with sarcomere length control yielded quantitatively similar results (see Fig. 8). Force redevelopment traces in a representative skinned psoas fiber at maximal and submaximal levels of Ca^{2+} activation are shown in Fig. 1: the top panel depicts the mechanical release-restretch maneuver imposed to determine the rate of force redevelopment (k_{tr}); and the bottom panel illustrates changes in submaximal force in solutions of pCa 6.1 (trace b) and pCa 6.0 (trace c) under control conditions. The change in force is expressed relative to maximum force (P_o) generated by the same fiber under control conditions when exposed to solution of pCa 4.5 solution (trace a).

Force-pCa Relationship. During measurements of k_{tr}, each skinned psoas fiber was exposed to solutions of varying pCa and allowed to develop steady-state force. The difference between steady-state force and the force baseline after the slack step was measured as the total force at that pCa. Active force was obtained by subtracting Ca^{2+}-independent force, measured in solution of pCa 9.0, from the total force. Force-pCa relationships were determined by expressing submaximal force (P) at each pCa as a fraction of maximal force (P_o) determined at pCa 4.5, i.e., P/P_o. The apparent cooperativity in the activation of force development was inferred from the steepness of the force-pCa relationship at forces <0.50 P_o, and was quantified using a Hill plot transformation of the force-pCa data (Strang et al., 1994). We focused on this region of the curve because the force-pCa relationship is biphasic, with most of the cooperative activation evident at forces less than 0.50 P_o (Moss, 1992). The force-pCa data were fit using the equation, $P/P_o = [Ca^{2+}]^n/(k^n + [Ca^{2+}]^n)$, where n is the Hill coefficient, and k is the $[Ca^{2+}]$ required for half-maximal activation.

Quantification of Partial TnC Extraction by SDS-PAGE

The extent of TnC extraction was assessed using SDS-PAGE and ultrasensitive silver staining (Sweitzer and Moss, 1993). Upon

completion of a given experiment, $\sim$0.75 mm of fiber segment was placed in a microfuge tube containing SDS sample buffer (10 μl/mm segment length) and stored at $-80°$C until analyzed for TnC content. The proportion of TnC present in the fiber was determined by densitometric analysis of silver-stained gels using a
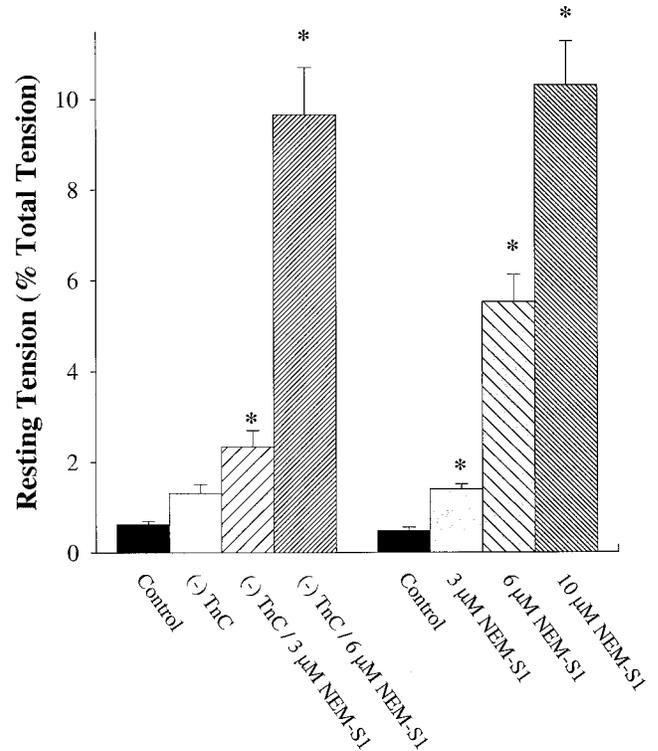

FIGURE 2. Effect of partial TnC extraction and NEM-S1 treatment on resting tension. Values are mean $\pm$ SEM, with numbers of fibers per treatment listed in Table I. $(-)$ TnC indicates partial TnC extraction. (Asterisk) Statistical difference experimental groups versus appropriate control groups, $P < 0.05$.

GS-670 imaging densitometer and Molecular Analyst software (BioRad Laboratories). To quantify the amount of TnC extracted, the ratio of TnC/(MLC1 + MLC3) was determined for each fiber (Moss, 1992). The ratio measured in the extracted fiber segment was divided by the ratio obtained from a segment obtained from the same fiber before extraction to yield an estimate of the TnC content of the partially TnC-extracted segment.

Statistics

All data are expressed as mean ± SEM. Where appropriate, either a two-tailed t test for independent samples or a paired t test was used as a post hoc test of significance, with significance set at $P < 0.05$.

RESULTS

The Effects of NEM-S1 and Partial Extraction of TnC on Steady-state Mechanical Properties

Our studies of cooperative mechanisms in skinned skeletal muscle fibers involved two perturbations that previously have been shown to alter cooperation in the activation of contraction. NEM-S1 was used to mimic the effects of strong-binding cross-bridges to further activate Ca^{2+}-dependent tension and the rate of tension development (Swartz and Moss, 1992), and thin filaments were partially TnC-extracted to disrupt near-neighbor cooperativity between adjacent functional groups (one troponin, one tropomyosin, and associated actins) in the thin filament (Brandt et al., 1984; Moss et al., 1985).

Treatment with NEM-S1. NEM-S1 had pronounced effects on maximal Ca^{2+}-activated tension (P_o), Ca^{2+}-independent tension, Ca^{2+} sensitivity of tension (pCa_{50}), and Hill coefficient (n_2) in single skinned psoas fibers, as reported earlier (Swartz and Moss, 1992) and summarized in Table I. The application of 3, 6, or 10 μM NEM-S1 increased Ca^{2+}-independent tension at pCa 9.0 in a concentration-dependent manner (Fig. 2). At the two highest concentrations used, the increase in Ca^{2+}-independent tension was accompanied by small (at 6 μM) or large (at 10 μM) reductions in maximal Ca^{2+}-activated tension, indicating that at these concentrations NEM-S1 competed with endogenous cross-bridges for binding sites on actin. For this reason, 6 μM NEM-S1 was preferentially used in most of the experiments described below. The increases in Ca^{2+}-independent tension are most likely due to cooperative activation of the thin filament by NEM-S1, thereby allowing strong binding of endogenous cross-bridges (Swartz and Moss, 1992).

NEM-S1 also potentiated submaximal Ca^{2+}-activated force in a concentration-dependent manner (Fig. 3). Mean pCa_{50} was significantly increased after treatment with either 6 μM NEM-S1 ($\Delta pCa_{50} = 0.07 \pm 0.01$, $P < 0.05$) or 10 μM NEM-S1 ($\Delta pCa50 = 0.12 \pm 0.03$, $P < 0.05$), so that Ca^{2+} sensitivity of force was increased. Such increases are consistent with the idea that, at low levels of Ca^{2+}, NEM-S1 promotes the formation of strongly bound, force-generating cross-bridges (Swartz

and Moss, 1992). At higher Ca^{2+} concentrations, i.e., pCa < 5.9, NEM-S1 had small or negligible effects on Ca^{2+}-activated forces relative to the control. Commensurate with the increase in force at low levels of Ca^{2+}, NEM-S1 significantly reduced the slope of the force-pCa relationship for Ca^{2+}-activated tensions <0.50 P_o (n_2), an effect that increased with increasing concentrations of NEM-S1 (Fig. 3 B and Table I).

Treatment with NEM-S1 and Partial Extraction of TnC. To further examine the mechanisms of cooperative activation of thin filaments, single fibers were first incubated with 200 μM TFP to partially extract TnC and
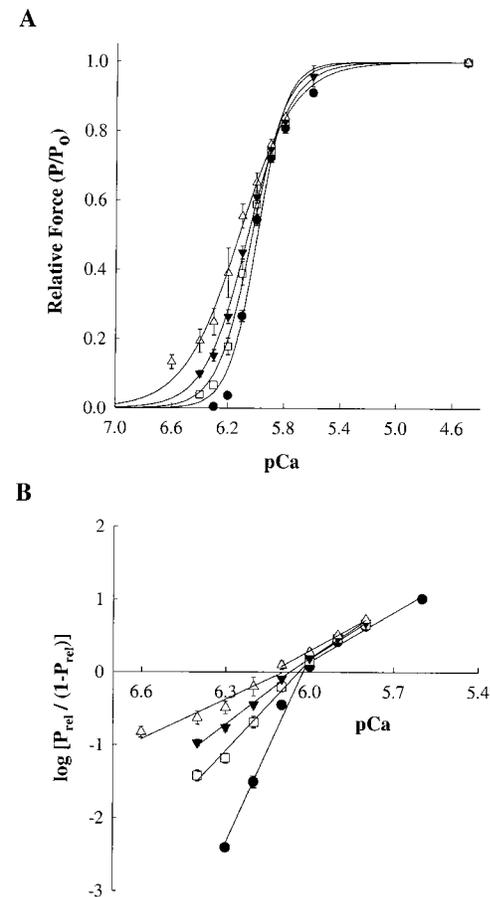

FIGURE 3. Effect of NEM-S1 on the force-pCa relationships in skinned psoas fibers. Values are means ± SEM. Fiber characteristics are listed in Table I. (A) Force-pCa relationships were determined in control fibers and in the same fibers after exposure to NEM-S1. Smooth lines were generated by fitting the mean data with the Hill equation: $P/P_o = [Ca^{2+}]^n/(k^n + [Ca^{2+}]^n)$, where n is the Hill coefficient, and k is the $[Ca^{2+}]$ required for half-maximal activation (i.e., pCa_{50}). pCa_{50} values from skinned fibers in the absence and presence of NEM-S1 were as follows: control (●), 6.00 ± 0.01; 3 μM NEM-S1 (□), 6.04 ± 0.02; 6 μM NEM-S1 (▼), 6.06 ± 0.02; and 10 μM NEM-S1 (△), 6.12 ± 0.04. (B) Hill plot transformations of the tension-pCa data were generated using the following equation: $\log[P_{rel}/(1 - P_{rel})] = n(\log[Ca^{2+}] + k)$, where P_{rel} is force as a fraction of P_o, n is the Hill coefficient, and k is the $[Ca^{2+}]$ required for half-maximal activation (i.e., pCa_{50}).
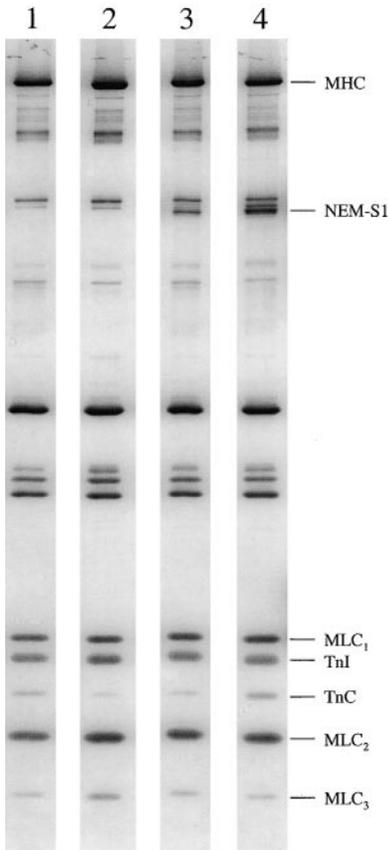
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FIGURE 4. Troponin C content of single psoas fibers after partial extraction of TnC. 12% SDS-PAGE of single-skinned psoas fibers before TnC extraction (lane 1), after partial TnC extraction (lanes 2 and 3), and after readdition of skeletal TnC to a TnC-extracted fiber (lane 4). To quantify the amount of TnC extracted, the ratio [TnC/(LC$_1$ + LC$_2$)] was determined for control, extracted, and reconstituted fiber segments by measuring the areas under the densitometric peaks corresponding to these proteins. The ratios from extracted and reconstituted fiber segments were divided by the ratio from a control segment of the same fiber to determine TnC content. Approximately 50% of endogenous TnC was extracted (lane 2 and 3) from these fibers, whereas readdition of skeletal TnC was stoichiometric (lane 4). Comparisons of the [MLC$_2$/(MLC$_1$ + MLC$_3$)] ratios revealed no alterations in MLC$_2$ content after partial TnC extraction.

were subsequently treated with NEM-S1. Such an approach allows dissection of the relative roles of cross-bridge binding and near-neighbor interactions in the thin filament in activation of contraction. In these experiments, segments of each fiber were analyzed by SDS-PAGE (Fig. 4) before TnC extraction (lane 1), after partial TnC extraction (lane 2), after TnC extraction plus NEM-S1 (lane 3), and after readdition of skeletal TnC to a previously extracted fiber (lane 4). Densitometric scans of the gels indicated that ~50% of the endogenous TnC was extracted from psoas fibers under the conditions used. Furthermore, the extraction procedure was specific for TnC since the relative
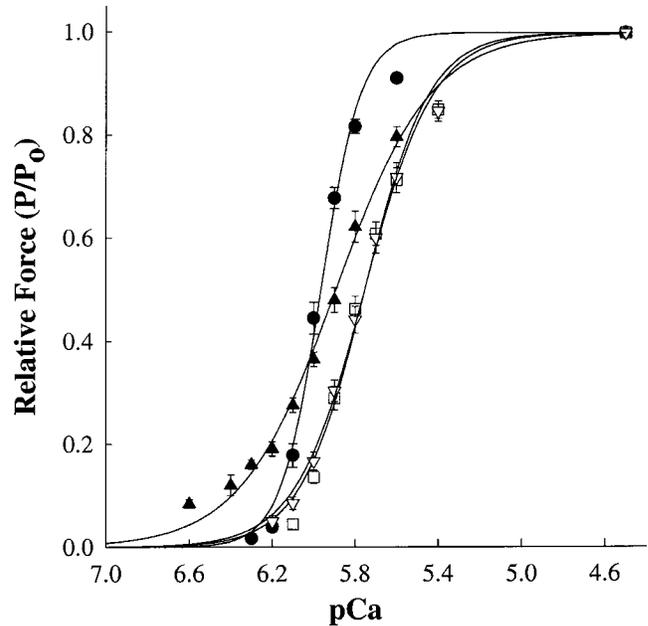

FIGURE 5. Effects of partial TnC extraction and NEM-S1 on force-pCa relationships in skinned psoas fibers. Values are means ± SEM. Force-pCa relationships were determined in control fibers and then in the same fibers after treatment with NEM-S1. Smooth lines were generated by fitting the mean data with the Hill equation: $P/P_o = [Ca^{2+}]^n/(k^n + [Ca^{2+}]^n)$, where n is the Hill coefficient, and k is the $[Ca^{2+}]$ required for half-maximal activation (i.e., pCa$_{50}$). pCa$_{50}$ values from skinned fibers under control conditions, after partial TnC extraction ((−) TnC) and after partial TnC and NEM-S1 treatment were as follows: control (●), 5.97 ± 0.01; (−) TnC (□), 5.75 ± 0.02; (−) TnC/3 μM NEM-S1 (▽), 5.76 ± 0.02; and (−) TnC / 6 μM NEM-S1 (▲), 5.89 ± 0.02. Fiber characteristics are listed in Table I.

amounts of other myofibrillar proteins (e.g., myosin light chains, TnI, and TnT) were unchanged.

Partial extraction of TnC had reversible effects on maximal Ca^{2+}-activated tension (P$_o$), Ca^{2+}-independent tension, Ca^{2+} sensitivity of tension, and the Hill coefficient (n$_2$), which are summarized in Table I. As reported previously (Brandt et al., 1984, 1990; Moss et al., 1985; Brandt and Schachat, 1997), partial extraction of TnC resulted in a significant decrease in maximum Ca^{2+}-activated tension, presumably because of the inactivation of thin filament functional groups. Subsequent treatment of partially TnC-extracted fibers with NEM-S1 resulted in a further reduction in maximal Ca^{2+}-activated tension similar to that observed after NEM-S1 treatment alone. Although resting tension was unaffected by partial TnC extraction alone, TnC extraction elicited significant increases in NEM-S1–induced Ca^{2+}-independent tension (Fig. 2). The increase in Ca^{2+}-independent tension, as the percent maximum force in untreated fibers, was less than that observed because of NEM-S1 treatment alone, suggesting that TnC extraction reduced the number of NEM-S1 or endogenous cross-bridges bound to actin, perhaps by disrupting

TABLE II

Summaries of k_{tr} and Ca^{2+}-activated Force at Maximal and Submaximal Concentrations of Ca^{2+} after Partial Tnc Extraction and Treatment with NEM-S1 in Skinned Psoas Fibers

	Control(15)	(−) TnC (15)	(−) TnC/3 μM NEM-S1 (7)	(−) TnC/6 μM NEM-S1 (8)
pCa 4.5				
k_{tr}	14.3 ± 0.8	14.8 ± 0.4	17.3 ± 0.7	18.7 ± 0.5*‡
pCa 6.0				
P/P$_o$	0.45 ± 0.03	0.14 ± 0.01	0.17 ± 0.02*	0.37 ± 0.01‡
k_{tr}	4.2 ± 0.2	3.2 ± 0.2	11.0 ± 1.4*‡	18.9 ± 1.0*‡
relative k_{tr}	0.30 ± 0.02	0.22 ± 0.02	0.63 ± 0.06*‡	1.02 ± 0.02*‡
	Control (18)	3 μM NEM-S1 (7)	6 μM NEM-S1 (8)	10 μM NEM-S1 (3)
pCa 4.5				
k_{tr}	13.6 ± 0.5	13.5 ± 0.7	13.4 ± 0.59	13.2 ± 0.7
pCa6.1				
P/P$_o$	0.27 ± 0.02	0.39 ± 0.03	0.45 ± 0.02*	0.55 ± 0.04*
k_{tr}	1.9 ± 0.1	7.2 ± 0.4*	9.5 ± 0.6*	11.8 ± 1.2*
relative k_{tr}	0.14 ± 0.01	0.56 ± 0.05*	0.70 ± 0.06*	0.88 ± 0.01*

All values are expressed as means ± SEM, with the number of skeletal muscle fibers given in parentheses. (−) TnC, partial TnC extraction; P/P$_o$, relative Ca^{2+}-activated force; k_{tr}, rate of tension redevelopment in seconds^{-1}; relative k_{tr}, the relative rate of force redevelopment. Both P/P$_o$ and relative k_{tr} values obtained in pCa 6.0 and pCa 6.1 were normalized to maximal values in pCa 4.5 under the same conditions (i.e., partial TnC extraction, partial TnC extraction and NEM-S1 treatment, or NEM-S1 treatment).
*Significantly different from the control.
‡Significantly different from (−) TnC fibers.

near-neighbor interactions between adjacent functional groups. However, when scaled to the maximum force after TnC extraction, the combined effects of TnC extraction and NEM-S1 were greater than with NEM-S1 alone.

Partial extraction of TnC also affected the Ca^{2+} sensitivity of tension and reduced the responsiveness of fibers to NEM-S1 (Fig. 5). Mean pCa$_{50}$ in control fibers was 5.97 ± 0.01, which decreased to 5.75 ± 0.02 after extraction of TnC (ΔpCa$_{50}$ = 0.22 ± 0.03, $P < 0.05$). Subsequent treatment with 3 μM NEM-S1 did not alter the Ca^{2+} sensitivity of force (ΔpCa$_{50}$ = 0.01 ± 0.01); however, 6 μM NEM-S1 increased the Ca^{2+} sensitivity of force, i.e., pCa$_{50}$ increased to 5.89 ± 0.02 (ΔpCa$_{50}$ = 0.15 ± 0.01; $P < 0.05$), a mean value near that observed in TnC-replete fibers when treated with NEM-S1 (Table I). Partial extraction of TnC and successive treatment with increasing concentrations of NEM-S1 resulted in progressive reductions in the steepness of the force-pCa relationship (Table I), suggesting that the effects of these two treatments on cooperativity are additive and most likely involve different molecular mechanisms.

Effects of NEM-S1 and Partial Extraction of TnC on the Rate of Force Redevelopment

According to Campbell's (1997) model of regulation, cooperative interactions within the thin filament, such as cross-bridge–induced cross-bridge binding, are thought to speed the rate of force redevelopment (k_{tr}), and may provide the basis for the steep activation dependence of k_{tr} in the skeletal muscle fibers observed

by Brenner (1988) and others. Previous work in which NEM-S1 was found to accelerate k_{tr} at all submaximal levels of Ca^{2+} activation (Swartz and Moss, 1992) provided strong support for this idea. However, the picture that emerged from that study was incomplete since in the presence of NEM-S1, k_{tr} was maximal at low and high levels of activation but less than maximal at intermediate levels of activation. Therefore, we have done additional experiments in an attempt to determine the basis for activation dependence of k_{tr} in the presence of NEM-S1 (data are summarized in Table II). This was done by examining the activation dependence of k_{tr} in the presence of varying amounts of NEM-S1 and also by partial extraction of TnC to perturb cooperative interactions within the thin filament.

Consistent with earlier results, k_{tr} was found to vary ~10-fold as Ca^{2+} activation was varied from near threshold to maximal levels. As shown in Fig. 6 A for a single submaximal pCa (pCa 6.1), the rate of force redevelopment at submaximal concentrations of Ca^{2+} was accelerated by NEM-S1, with the degree of acceleration increasing with the concentration of NEM-S1 (Fig. 6 A, traces b–d), compared with control (trace a). Under identical experimental conditions, another fiber was subjected to partial TnC extraction and subsequent treatment with 6 μM NEM-S1 (Fig. 6 B). As summarized previously, k_{tr} under control conditions varied with the level of Ca^{2+} activation, increasing when Ca^{2+} concentration was increased from pCa 5.9 (Fig. 6 B, trace b) to pCa 4.5 (Fig. 6 B, trace a). After partial extraction of TnC, the steady force decreased at each
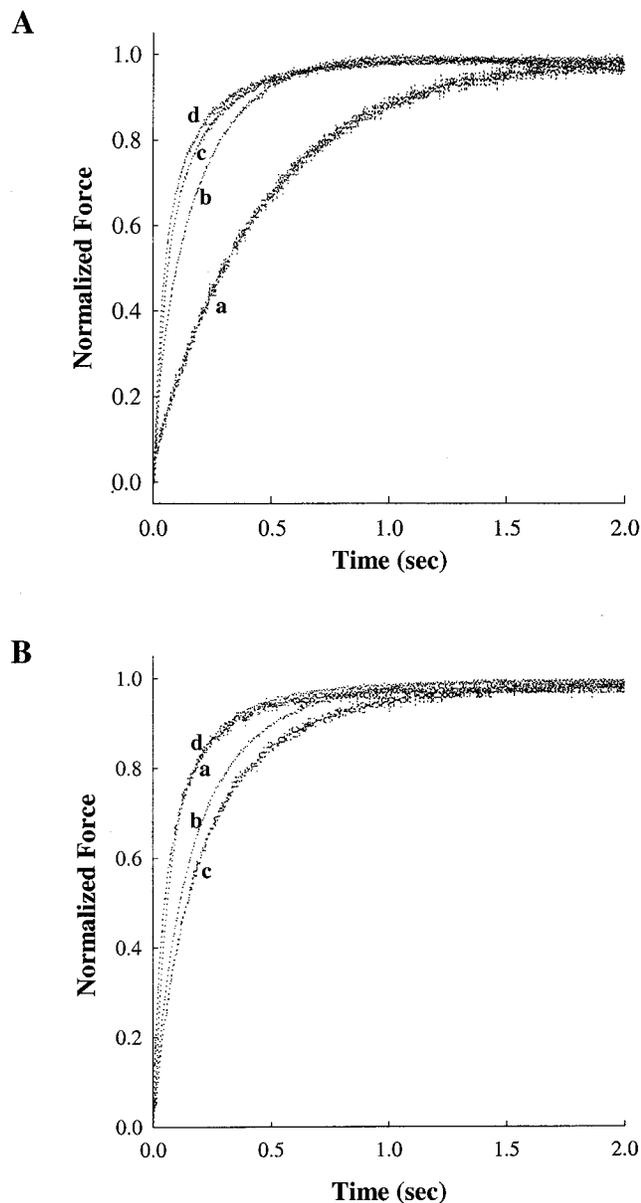
FIGURE 6. Effects of reduced cooperativity of activation on the rate of force redevelopment. Differences in the rate constant of force redevelopment (k_{tr}) were expressed relative to respective peak forces. (A) The rates of force redevelopment at pCa 6.1 in skinned psoas fibers in the absence (trace a, $P/P_o = 0.20$, $k_{tr} = 2.2$ s^{-1}) and presence of 3 μM NEM-S1, (trace b, $P/P_o = 0.41$, $k_{tr} = 7.1$ s^{-1}), 6 μM NEM-S1 (trace c, $P/P_o = 0.47$, $k_{tr} = 12.6$ s^{-1}), or 10 μM NEM-S1 (trace d, $P/P_o = 0.53$, $k_{tr} = 14.1$ s^{-1}). Maximal k_{tr} was 15.1 s^{-1} for the fiber in traces a and c, 15.8 s^{-1} for the fiber in trace b, and 15.4 s^{-1} for the fiber in trace d. (B) The rates of force redevelopment in skinned psoas fibers incubated in solutions of pCa 4.5 (trace a, $P/P_o = 1.0$, $k_{tr} = 11.4$ s^{-1}) and pCa 5.9 under control conditions (trace b, $P/P_o = 0.64$, $k_{tr} = 5.9$ s^{-1}), after partial TnC extraction (trace c, $P/P_o = 0.32$, $k_{tr} = 5.1$ s^{-1}), and after the combined treatment of partial TnC extraction and 6 μM NEM-S1 (trace d, $P/P_o = 0.48$, $k_{tr} = 18.7$ s^{-1}). The relative force values obtained at pCa 5.9 were normalized to 1.0 to allow comparisons between the various experimental conditions, i.e., control, TnC-extracted or TnC-extracted/NEM-S1.
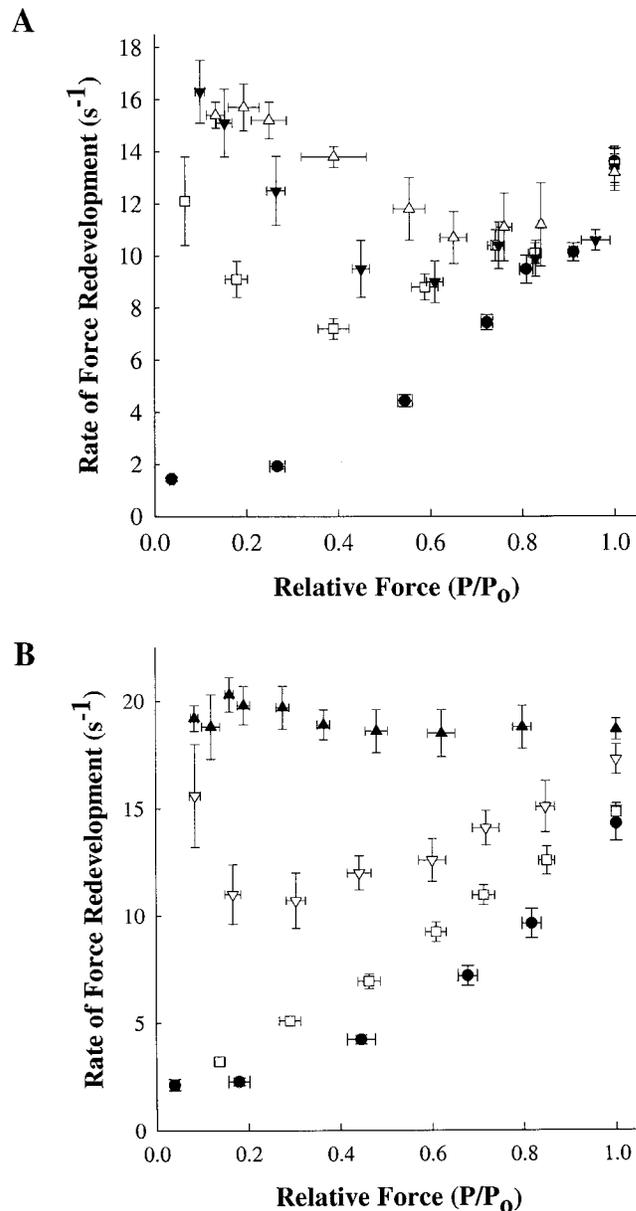
FIGURE 7. Alteration in the activation dependence of the rate of force redevelopment after partial extraction of TnC and treatment with NEM-S1. Force redevelopment following the release-restretch mechanical maneuver was recorded as a function of relative steady-state isometric force (P/P_o) developed before the maneuver. All values are means ± SEM. (A) NEM-S1–induced effects on the force dependence of k_{tr} in the absence (●) and presence of 3 μM NEM-S1 (□), 6 μM NEM-S1 (▼), and 10 μM NEM-S1 (△). (B) Force dependence of k_{tr} in fibers under control conditions (●), after partial TnC extraction (□), partial TnC extraction, and after treatment with 3 μM NEM-S1 (▽), and partial TnC extraction and treatment with 6 μM NEM-S1 (▲).
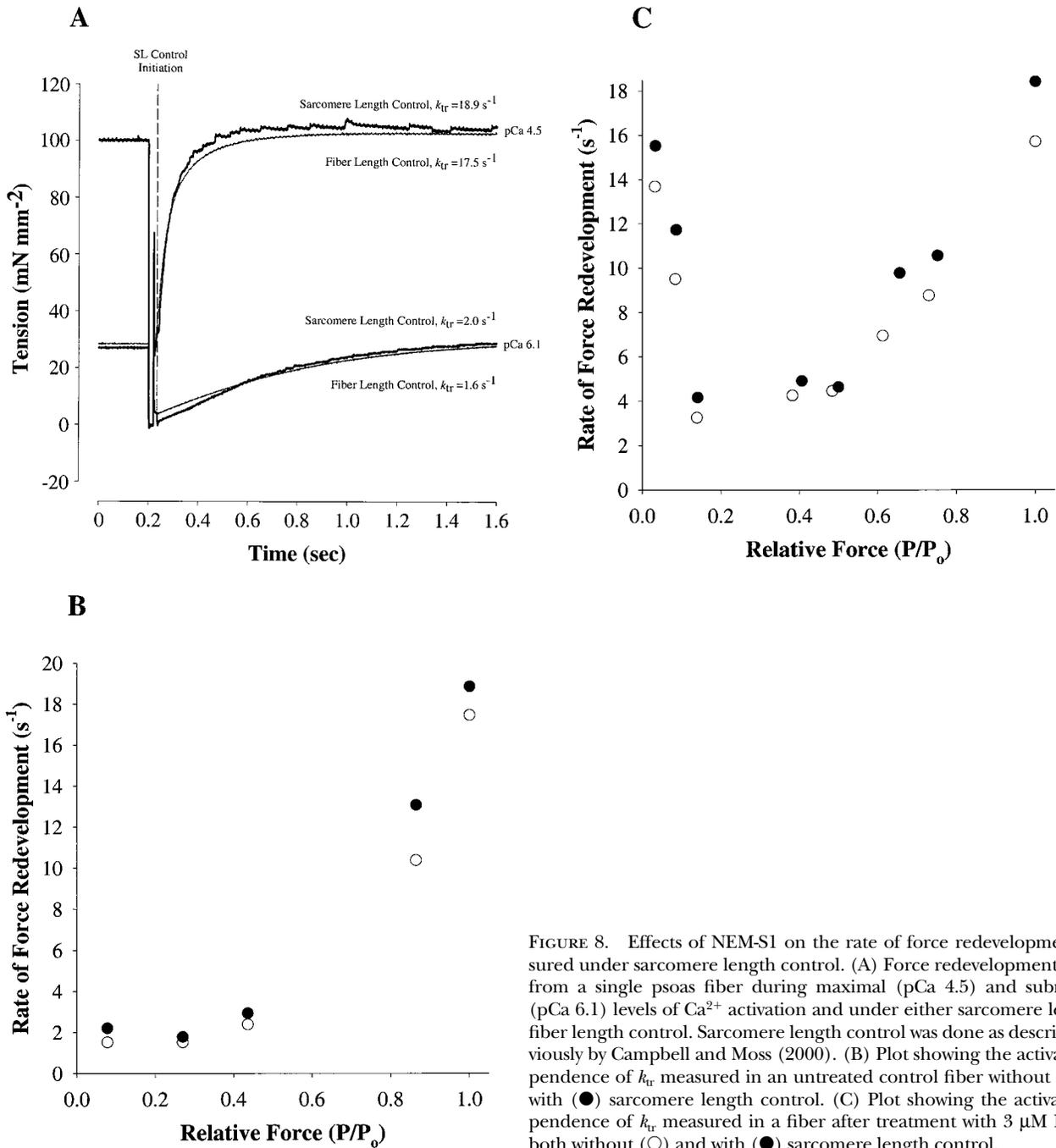
A

B

C

FIGURE 8. Effects of NEM-S1 on the rate of force redevelopment measured under sarcomere length control. (A) Force redevelopment records from a single psoas fiber during maximal (pCa 4.5) and submaximal (pCa 6.1) levels of Ca^{2+} activation and under either sarcomere length or fiber length control. Sarcomere length control was done as described previously by Campbell and Moss (2000). (B) Plot showing the activation dependence of k_{tr} measured in an untreated control fiber without ($\bigcirc$) and with ($\bullet$) sarcomere length control. (C) Plot showing the activation dependence of k_{tr} measured in a fiber after treatment with 3 μM NEM-S1, both without ($\bigcirc$) and with ($\bullet$) sarcomere length control.

pCa, but the rate of force redevelopment (Fig. 6 B, trace c) at pCa 5.9 was nearly equivalent to that of the preextracted fiber at the same pCa. Subsequent treatment with 6 μM NEM-S1 significantly increased steady force at pCa 5.9 and markedly accelerated the rate of force redevelopment (Fig. 6 B, trace d).

The relationship between k_{tr} and steady-state isometric force (as a measure of the level of activation due to Ca^{2+} binding and cooperative mechanisms) was variably affected by interventions to increase numbers of strongly bound cross-bridges (i.e., NEM-S1) and to dis-

rupt near-neighbor cooperativity in the thin filament (partial extraction of TnC). As shown in Fig. 7 A, k_{tr} during maximal activation was unaffected by NEM-S1, but k_{tr} values at low levels of activation were increased to levels either identical to (at 3 μM NEM-S1) or greater than (at 6 or 10 μM NEM-S1) the values obtained in maximally activated fibers. At intermediate levels of activation, NEM-S1 increased k_{tr} to greater than the control values, but k_{tr} was still less than maximal. Similar effects of NEM-S1 were observed in fibers that were subjected to sarcomere length control during measurements of k_{tr}
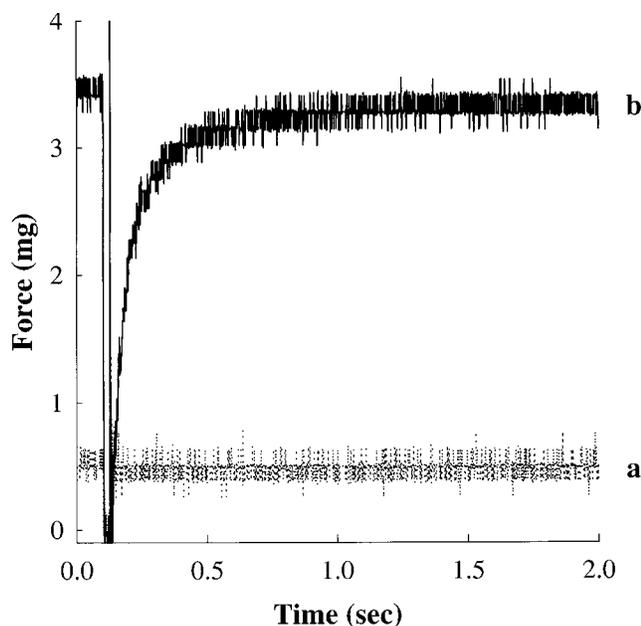
FIGURE 9. Force redevelopment time course at pCa 9.0 in NEM-S1–treated fiber. Ca²⁺-independent force and force redevelopment in a representative skinned psoas fiber in skinned single fibers in the absence (trace a) and presence of 10 μM NEM-S1 (trace b). Force at pCa 4.5 (P_o) in the absence of NEM-S1 was 139 mN/mm²; k_{tr} under the same conditions was 12.8 s⁻¹. Ca²⁺-independent force in the absence of NEM-S1 was 0.8% P_o; Ca²⁺-independent force in the presence of NEM-S1 was 8.5% P_o; k_{tr} in the presence of NEM-S1 was 19.3 s⁻¹.

(Fig. 8). Progressive increases in the concentration of NEM-S1 further increased the value of k_{tr} at intermediate levels of activation, but even the highest concentration used (10 μM) did not accelerate k_{tr} to maximal values. In the absence of Ca²⁺ (pCa 9.0), application of

NEM-S1 induced active tension development (Fig. 9 and Table III); under these conditions, k_{tr} was supramaximal when compared with the maximal values measured at pCa 4.5 under control conditions.

Partial extraction of TnC also increased k_{tr} at intermediate levels of activation as compared with the control (Fig. 7 B). In fact, the increase in k_{tr} varied with the extent of TnC extraction, i.e., fibers containing smaller amounts of residual TnC exhibited faster rates of force redevelopment during submaximal activation (Fig. 10). Subsequent treatment of partially TnC extracted fibers with 3 μM NEM-S1 resulted in a k_{tr}–relative force relationship similar to that observed with NEM-S1 treatment alone, although k_{tr} at each level of activation was somewhat greater than the value obtained with NEM-S1 alone. However, the combination of partial TnC extraction and 6 μM NEM-S1 increased k_{tr} at each level of activation to values greater than the maximum measured in control fibers and completely eliminated the activation dependence of k_{tr} (Fig. 7 B).

DISCUSSION

The results of the present study show that strong binding of cross-bridges to actin increases the rate of force development in skeletal muscle and suggest that cooperativity in cross-bridge binding is an important determinant of activation kinetics. NEM-S1 increased the k_{tr} measured during submaximal activations, with greatest effects at low levels of activation where NEM-S1 increased k_{tr} to values greater than the maximum observed in control fibers at saturating [Ca²⁺]. From this result, it is evident that the rate of force development measured in control fibers at pCa 4.5 is not a true maxi-

TABLE III

Summaries of k_{tr} in Solution of pCa after Partial TnC Extraction and Treatment with NEM-S1 in Skinned Psoas Fibers

	Control (15)	(−) TnC (15)	(−) TnC/3 μM NEM-S1 (7)	(−) TnC/6 μM NEM-S1 (8)
pCa 4.5				
k_{tr}	14.3 ± 0.8	14.8 ± 0.4	17.3 ± 0.7	18.7 ± 0.5*
pCa 9.0				
k_{tr}				19.0 ± 0.5
relative k_{tr}				1.02 ± 0.04

	Control (18)	3 μM NEM-S1 (7)	6 μM NEM-S1 (8)	10 μM NEM-S1 (3)
pCa 4.5				
k_{tr}	13.6 ± 0.5	13.5 ± 0.7	13.4 ± 0.59	13.2 ± 0.7
pCa 9.0		18.4 ± 1.0‡	19.4 ± 1.0‡	18.2 ± 0.8‡
k_{tr}		1.34 ± 0.12	1.37 ± 0.13	1.39 ± 0.04

All values are expressed as means ± SEM, with the number of skeletal muscle fibers given in parentheses. (−) TnC, partial TnC extraction; P/P_o, relative Ca²⁺-activated force; k_{tr}, rate of tension redevelopment in seconds⁻¹; relative k_{tr}, the relative rate of force redevelopment. Relative k_{tr} values obtained in pCa 9.0 were normalized to maximal values in pCa 4.5 under the same conditions (i.e., partial TnC extraction, partial TnC extraction and NEM-S1 treatment, or NEM-S1 treatment).

*Significantly different from the control.

‡Significantly different from maximal values in pCa 4.5 under the same conditions.

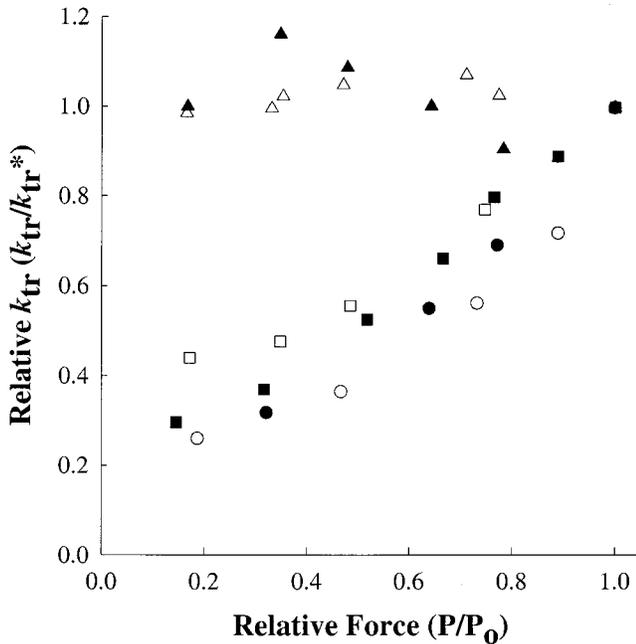Downloaded from http://rupress.org/jgp/article-pdf/117/2/133/1194046/8209.pdf by guest on 01 July 2022

FIGURE 10. Effects of variable extraction of TnC on the activation dependence of k_{tr}. The relationship between k_{tr} and the relative force is presented for two distinct fibers, 2801B (open symbols) and 2901B (closed symbols), under control conditions (○, ●) after partial TnC extraction (□, ■), and after partial TnC extraction and treatment with 6 μM NEM-S1 (△, ▲). Varying the extent of TnC extraction (by reducing the incubation time in extracting solution) reduced the maximal force to 0.15 P_o in fiber 2801B and to 0.65 P_o in fiber 2901B.

mum. Although NEM-S1 dramatically accelerated k_{tr}, strong binding of cross-bridges does not entirely account for the activation of cross-bridge kinetics, since NEM-S1 alone was insufficient at intermediate levels of activation to increase k_{tr} to maximal or to completely eliminate the activation dependence of k_{tr}. Instead, elimination of activation dependence required both NEM-S1 and partial extraction of TnC from the thin filament. Since partial extraction of TnC should disrupt near-neighbor communication between functional groups in the thin filament (Brandt et al., 1984, 1990; Moss et al., 1985), we conclude that activation dependence of k_{tr} results from effects of strongly bound cross-bridges to cooperatively recruit additional cross-bridges within the same and neighboring regions of the thin filament.

Models for Activation of Contraction

Ca^{2+} binding to TnC initiates muscle contraction, but complete activation of tension and the kinetics of tension development appears to involve cooperative effects due to cross-bridge binding to actin (for reviews see Lehrer, 1994; Gordon et al., 2000). Biochemical data suggest that activation is a positive cooperative process (Williams et al., 1984, 1988), such that activation of a thin filament functional group (estimated to

be ± 10–14 actin monomers; Geeves and Lehrer, 1994; Swartz et al., 1996) by Ca^{2+} and/or strong binding cross-bridges influences the activation of neighboring functional groups (Lehrer, 1994). Cooperation is apparent in contracting muscle in the greater than expected (on the basis of the numbers of Ca^{2+} binding sites on TnC) steepness of the tension-pCa relationship, especially at low levels of Ca^{2+} (for review see Moss, 1992), and previously observed effects of strong-binding cross-bridges to speed the rate of submaximal force development in skinned skeletal muscle fibers (Swartz and Moss, 1992).

The rate constant of force redevelopment (k_{tr}) in steadily activated skeletal muscle preparations exhibits an ∼10-fold activation as [Ca^{2+}] is increased from threshold to saturating levels, which was originally shown by Brenner and Eisenberg (1986) and, subsequently, has been reported by other groups. Brenner (Brenner, 1988; Brenner and Chalovich, 1999) has explained the activation dependence of k_{tr} using a model in which Ca^{2+} influences the rate constant of cross-bridge attachment to actin, f_{app}, i.e., f_{app} decreases when [Ca^{2+}] is lowered, presumably by an allosteric mechanism. Such a model does not straightforwardly account for our observation that strong-binding cross-bridges (NEM-S1) markedly accelerate k_{tr}, an effect which is greatest at low levels of Ca^{2+} activation: but, our results don't exclude the possibility that this type of mechanism modulates kinetics in control fibers. A simple explanation is that increased [Ca^{2+}] effectively increases f_{app} as a consequence of the cooperative effects of increased numbers of cross-bridges bound to the thin filament.

At least two models of activation might be used to explain the activation dependence of k_{tr}. Landesberg and Sideman (1994) developed a model in which the activation dependence of the rate of force development in cardiac muscle was due to a cross-bridge binding–dependent increase in Ca^{2+} binding affinity of TnC. Alternatively, the steep activation dependence of k_{tr} can be explained by the cooperation-mediated slowing of force development at low levels of activation (Campbell, 1997), which is the framework we will use to discuss our results. In Campbell's model, cross-bridges are distributed between cycling and noncycling populations: cycling cross-bridges undergo repeated transitions between non–force-bearing and force-bearing states (under the influence of the rate constants f_{app} and g_{app}), whereas noncycling cross-bridges are recruited to the cycling population as a result of Ca^{2+} binding to troponin or cooperative effects of strong binding cross-bridges to enhance activation of the thin filament. At lower levels of Ca^{2+}, a smaller fraction of cross-bridges is initially recruited into the cycling population as a direct result of Ca^{2+} binding to the thin filament, so that most cross-bridges are in the noncycling

Double Exponential Phases of Force Redevelopment in Skinned Single Psoas Fibers after Partial TnC Extraction and Treatment with NEM-S1

Group	P/P_o	a	k_1	b	k_2
Control (15)	0.23 ± 0.02	0.48 ± 0.08	4.8 ± 0.8	0.56 ± 0.06	2.0 ± 0.1
	0.54 ± 0.01	0.63 ± 0.08	7.0 ± 0.6	0.46 ± 0.07	3.6 ± 0.3
	1.0	0.74 ± 0.01	20.4 ± 1.3	0.25 ± 0.01	3.9 ± 0.2
$(-)$ TnC (15)	0.22 ± 0.02	0.27 ± 0.05	10.9 ± 1.0	0.65 ± 0.05	2.8 ± 0.2
	0.54 ± 0.01	0.38 ± 0.04	17.3 ± 1.7	0.58 ± 0.04	4.3 ± 0.3
	1.0	0.51 ± 0.02	28.8 ± 1.7	0.47 ± 0.02	6.1 ± 0.3
$(-)$ TnC/3 μM NEM-S1 (7)	0.20 ± 0.01	0.45 ± 0.02	33.1 ± 4.0	0.44 ± 0.03	4.5 ± 0.5
	0.52 ± 0.02	0.39 ± 0.03	39.9 ± 6.0	0.57 ± 0.03	6.4 ± 0.5
	1.0	0.46 ± 0.03	32.1 ± 2.6	0.52 ± 0.02	7.2 ± 0.5
$(-)$ TnC/6 μM NEM-S1 (8)	0.21 ± 0.01	0.64 ± 0.02	37.0 ± 4.1	0.30 ± 0.02	4.6 ± 0.5
	0.51 ± 0.01	0.61 ± 0.02	35.1 ± 3.1	0.36 ± 0.02	5.0 ± 0.5
	1.0	0.63 ± 0.01	32.3 ± 1.3	0.36 ± 0.01	5.8 ± 0.4
Control (18)	0.25 ± 0.02	0.52 ± 0.09	2.2 ± 0.1	0.46 ± 0.08	1.9 ± 0.1
	0.58 ± 0.02	0.47 ± 0.10	8.3 ± 0.8	0.67 ± 0.08	4.3 ± 0.3
	1.0	0.73 ± 0.01	19.3 ± 1.0	0.26 ± 0.01	3.9 ± 0.2
3 μM NEM-S1 (7)	0.26 ± 0.02	0.35 ± 0.02	28.7 ± 3.5	0.62 ± 0.04	4.3 ± 0.4
	0.52 ± 0.03	0.26 ± 0.03	27.9 ± 3.3	0.73 ± 0.03	5.3 ± 0.4
	1.0	0.72 ± 0.01	18.7 ± 1.0	0.28 ± 0.01	3.6 ± 0.2
6 μM NEM-S1 (8)	0.27 ± 0.01	0.64 ± 0.03	25.5 ± 2.5	0.34 ± 0.03	4.4 ± 0.5
	0.47 ± 0.02	0.48 ± 0.04	27.1 ± 3.0	0.50 ± 0.03	5.2 ± 0.8
	1.0	0.74 ± 0.01	21.0 ± 0.01	0.26 ± 0.01	3.9 ± 0.3
10 μM NEM-S1 (3)	0.20 ± 0.02	0.71 ± 0.01	22.0 ± 0.8	0.27 ± 0.01	3.2 ± 0.2
	0.52 ± 0.01	0.59 ± 0.01	21.8 ± 2.2	0.40 ± 0.01	3.9 ± 0.4
	1.0	0.74 ± 0.01	18.7 ± 1.2	0.25 ± 0.01	3.3 ± 0.1

All values are expressed as means $\pm$ SEM, with the number of skeletal muscle fibers given in parentheses. Force redevelopment traces were fit with a double exponential equation, $y = a \cdot \exp(-k_1 \cdot x) + b \cdot \exp(-k_2 \cdot x)$, where a is the amplitude of the first exponential phase that rises with a rate constant of k_1 and b is the amplitude of the second exponential phase that rises with a rate constant of k_2.

pool and, thus, are available for cooperative recruitment to the cycling pool. Progressive recruitment of cross-bridges from the noncycling pool would then slow the rate of force development. In contrast, at high levels of Ca^{2+}, the rate constant of force development is much greater because most cross-bridges are recruited to the cycling pool when Ca^{2+} binds to troponin, which leaves few cross-bridges available in the noncycling pool for subsequent cooperative recruitment. At a saturating $[Ca^{2+}]$ of pCa 4.5, the rate constant of force development will thus be equal to $f_{app} + g_{app}$.

Activation Dependence of Force and k_{tr} Involves Activating Effects of Cross-bridge Binding and Near-neighbor Cooperativity within the Thin Filament

The results of our study provide support for models of regulation in which cross-bridge interaction kinetics are activated by cross-bridge binding to the thin filament. Experiments here show that force at submaximal $[Ca^{2+}]$ is increased due to activating effects of strongly bound cross-bridges, which confirms results from earlier studies from this and other laboratories (Swartz et

al., 1996 and references therein). The Ca^{2+} dependence of k_{tr} appears to involve two factors: activating effects due to cross-bridge binding to the thin filament, and near-neighbor interactions between adjacent functional groups, such that activation of a given functional group facilitates cross-bridge binding in neighboring functional groups. Thus, Ca^{2+} does not seem to regulate kinetics (e.g., by binding to a regulatory site) but, instead, has indirect effects on kinetics by influencing the number of cross-bridges bound to actin. Our results also show that cooperative effects due to cross-bridge binding to thin filaments are large but insufficient to completely account for activation dependence of k_{tr}, since it was necessary to add NEM-S1 and disrupt near-neighbor cooperativity in the thin filament to achieve maximal k_{tr} at all levels of activation.

Effects on Force and k_{tr} due to Partial Extraction of Troponin C. In this study, partial extraction of TnC was used to disrupt the spread of activation between adjacent functional groups in the thin filament, presumably because of constitutive inactivation of functional groups from which TnC was removed (Brandt et al., 1984; Moss et al., 1985; for review see Moss, 1992). Our find-

ings that the maximum force and the steepness of the force-pCa relationship were reduced by TnC extraction (Table I and Fig. 5) confirm earlier results (Brandt et al., 1984, 1990; Moss et al., 1985; Metzger and Moss, 1991) and support the idea that near-neighbor interactions between functional groups contribute to force development in skeletal muscle.

Whereas steady-state Ca^{2+}-activated force was reduced after TnC extraction, the rate constant of force redevelopment (k_{tr}) increased at each submaximal force, when compared with nonextracted fibers (Fig. 7 B). Although not anticipated in Campbell's (1997) model of activation, this result is consistent with the idea that cooperative interactions between thin filament functional groups contribute to a slowing of force development at low activation. By extracting TnC, these interactions were presumably disrupted and cooperative recruitment of cross-bridges from neighboring functional groups was reduced or eliminated, thereby speeding the rate of force development. By varying the amount of TnC extracted, it should be possible to vary the rate of force development independent of $[Ca^{2+}]$, which is what we observed. Fibers containing less TnC and, therefore, having fewer functional groups that could be activated by Ca^{2+} exhibited a faster rate of force redevelopment at each submaximal force (Fig. 10). Although it might be possible to explain these results by an alternate mechanism (e.g., direct effects on f_{app} due to extraction of TnC), a modification of Campbell's (1997) model to include near-neighbor interactions that cooperatively recruit cross-bridges to strongly bound states also accounts for our results (Razumova et al., 2000).

Effects on Force and k_{tr} due to NEM-S1. As reported earlier (Swartz and Moss, 1992), treatment of fibers with NEM-S1 reduced the steepness of the force-pCa relationship (Table I). This decrease in the apparent cooperativity of activation can be explained by near saturation of the myosin cross-bridge binding component of thin filament activation due to binding of NEM-S1. NEM-S1 also induced concentration-dependent increases in the resting (Ca^{2+}-independent) tension, submaximal Ca^{2+}-activated force, and the Ca^{2+} sensitivity of force, i.e., a left shift of the tension-pCa relationship (Table I and Fig. 3). Together, these results support the idea that binding of NEM-S1 to actin increased the level of thin filament activation and increased the number of endogenous cross-bridges in strongly bound force-generating states.

At submaximal levels of activation, NEM-S1 increased the rate of force redevelopment at each level of activation, but the increase was much greater at very low than at intermediate levels of activation. As the concentration of NEM-S1 was increased, k_{tr} at low levels of activation was increased to maximal and supramaximal values. One possible way to explain this phenomenon is

that at low levels of Ca^{2+}, the increased force and maximal values of k_{tr} are manifestations of preferential Ca^{2+} binding to functional groups in which NEM-S1 is also bound. The combined activating effects of Ca^{2+} and strongly bound cross-bridges within these functional groups would facilitate binding of endogenous cross-bridges and would accelerate the rate of binding. Although this is a plausible mechanism, it does not account for all of our results, since fibers activated with NEM-S1 in the absence of Ca^{2+} developed small forces but yielded k_{tr} values that were maximal or supramaximal. This finding indicates that k_{tr} measured in control fibers at pCa 4.5 is not the maximum value for this variable. Furthermore, it is possible to achieve maximal and even higher values of k_{tr} in the absence of Ca^{2+} binding to TnC (i.e., at pCa 9.0) simply by increasing the numbers of strong-binding cross-bridges in the form of NEM-S1. We don't know the mechanism of this effect, but it is possible that such high values of k_{tr} are due to a combination of NEM-S1 binding to a few discrete regions of the filament, and isolation of these regions from adjacent, inactive regions by intervening troponin complexes having no Ca^{2+} bound. The first condition would arise if the binding of NEM-S1 was not uniform along the thin filament, and would limit the number of endogenous cross-bridges that could bind to the thin filament, and thereby account for the small forces developed in the absence of Ca^{2+}. The second condition would reduce or eliminate communication between adjacent functional groups, eliminate near-neighbor cooperative recruitment of cross-bridges from the noncycling pool, and thereby speed k_{tr}. The fact that NEM-S1 does not further accelerate k_{tr} in maximally Ca^{2+}-activated fibers is consistent with both ideas, i.e., if NEM-S1 is nonuniformly distributed along the thin filament, then the maximum value of k_{tr} would be limited by cooperative recruitment of cross-bridges from adjacent functional groups with less or no NEM-S1 bound.

At intermediate levels of activation (indexed by force or $[Ca^{2+}]$), k_{tr} measured in the presence of NEM-S1 was less than that observed at low and maximal activations, but was much faster than control values measured at similar levels of force or Ca^{2+} (Fig. 7 A). The finding that k_{tr} in the presence of NEM-S1 is not maximal at all levels of activation suggests that the thin filament is not saturated by Ca^{2+}, cross-bridge binding, or by both. Again, it seems likely that NEM-S1 binding to the thin filament is not uniform, resulting in some regions with NEM-S1 bound and other regions with less or no NEM-S1. As discussed above, functional groups with NEM-S1 bound should be activated at lower levels of Ca^{2+}, because of a greater Ca^{2+} binding affinity, and the rate of force development would be maximal because of the combined effects of Ca^{2+} and bound cross-bridges. By similar reasoning, those with less or no NEM-S1 would

be recruited at higher (intermediate) levels of Ca^{2+} and will have fewer bound cross-bridges, i.e., cross-bridge activation of these functional groups is less, and force development is therefore slower. The fact that the records of force redevelopment at intermediate activation are well fit by a single rate constant suggests that the mix of variably activated functional groups confers a single level of activation to the entire thin filament. Thus, the effective size of a functional group appears to increase as $[Ca^{2+}]$ is increased to intermediate levels; the central region of the functional group has the greatest amounts of Ca^{2+} and NEM-S1 bound. Because of this, the rate of force development at intermediate levels of activation will be slowed because of cooperative recruitment of cross-bridges into the end regions of the functional group or adjacent functional groups. Ultimately, at high $[Ca^{2+}]$, fewer inactivated functional groups remain and the impact of cooperative interactions is reduced, but not totally eliminated since, in control fibers, k_{tr} never achieves the maximum possible value. Correspondingly, the time course of force redevelopment becomes progressively faster, and k_{tr} converges to the value obtained during maximal activation.

The effects of NEM-S1 to accelerate k_{tr} appear to be significantly greater than the effects due to partial extraction of TnC (Fig. 7 B). This result suggests that effects on kinetics due to cross-bridge binding within functional groups are greater than effects due to near-neighbor interactions between functional groups. Nevertheless, both mechanisms contribute to activation kinetics, since it was necessary to add NEM-S1 and partially extract TnC to eliminate the activation dependence of k_{tr}.

Effects due to Partial Extraction of TnC and Treatment with NEM-S1. Partial extraction of TnC or application of NEM-S1 has been shown to independently alter the Ca^{2+} activation of force in skeletal muscle, effects that are additive or synergistic. When fibers were treated in either way, the steepness of the force-pCa relationship was reduced (Table I), indicating a decrease in the apparent cooperativity of activation. Importantly, effects on steepness were much greater in fibers subjected to both interventions, i.e., extraction of 50% TnC and application of 6 μM NEM-S1, than with either alone. At present, we do not have a unique explanation for this result, but a simple model is one in which initial cross-bridge binding facilitates additional binding in the same and adjacent functional groups. In such a model, partial extraction of TnC would reduce steepness by disrupting near-neighbor interactions within the thin filament, and NEM-S1 would reduce steepness by cooperatively increasing the number of endogenous cross-bridges that bind within a functional group or in neighboring functional groups at each submaximal pCa.

The combination of TnC extraction and application

of NEM-S1 also had greater effects on the activation dependence of k_{tr} than either treatment alone. In fact, addition of 6 μM NEM-S1 to partially TnC-extracted fibers increased k_{tr} to greater than maximal values at all levels of activation and completely eliminated the activation dependence of k_{tr} (Fig. 7 B). These results strongly suggest that the slowing of k_{tr} observed in untreated fibers at low and intermediate levels of activation is due to cooperative binding of cross-bridges within the same and neighboring functional groups, and the acceleration of k_{tr} at high levels of activation reflects a decrease in the importance of such cooperation. This conclusion is consistent with Campbell's (1997) model of activation in which slowing of k_{tr} at low levels of activation involves progressive cooperative recruitment of cross-bridges from noncycling to cycling states. However, to account for the effects of TnC extraction to accelerate k_{tr}, this model must be expanded to include near-neighbor effects, i.e., cross-bridge binding in one functional group seems to cooperatively activate adjacent functional groups and thereby induce further cross-bridge binding (Razumova et al., 2000). In support of this idea, partial extraction of TnC was needed to completely eliminate the activation dependence of k_{tr}.

Consideration of Possible Artifacts in Experimental Measurements

Most measurements of k_{tr} in the present study were done without sarcomere length control, which has been shown previously to result in underestimation of the rate constant by as much as 50% because of mechanical effects of end compliance at the points of attachment to the fiber (Brenner, 1988). Such slowing, if undetected or variably present, could distort the results and affect our conclusions. Several lines of evidence suggest that this was not the case here. First, the mean maximum k_{tr} obtained in control fibers ($\sim$14 s^{-1} was $\sim$80% of the value [$\sim$18 s^{-1}] obtained in an earlier study using sarcomere length control; Metzger et al., 1989). The 10-fold variation in k_{tr} is similar to that observed under sarcomere length control. Second, under conditions that eliminated the activation dependence of k_{tr}, i.e., NEM-S1 plus partial extraction of TnC, k_{tr} was identical at all levels of activation regardless of force (Fig. 7 B), again indicating that variations in end compliance with developed force were not the basis for activation dependence of k_{tr}. Finally, in several fibers in which sarcomere length control was used, the effects of NEM-S1 on the activation dependence of k_{tr} were similar with (Fig. 8) and without (Fig. 7 A) sarcomere length control.

Another possibility is that NEM-S1 actually stiffens the fiber, thereby increasing k_{tr} at each level of activation. We regard this as highly unlikely for a couple of rea-

sons. First, in our previous measurement of k_{tr} (Metzger et al., 1989), sarcomere length clamping at very low levels of activation (P/P$_o$ = 0.14, pCa 6.2) resulted in k_{tr} values of ~1 s^{-1}. This is much lower than the values of k_{tr} measured in the present study in the presence of NEM-S1 (~16 s^{-1}) and with both NEM-S1 treatment and partial extraction of TnC (~19 s^{-1}). Second, treatment with NEM-S1 alone had no effect on k_{tr} in maximally activated fibers (k_{tr} = ~14 s^{-1}), but NEM-S1 plus partial extraction of TnC increased k_{tr} to ~19 s^{-1}.

Implications of Results for Regulation of Force and the Kinetics of Force Development under Physiological Conditions

Our results support the idea that Ca^{2+} activation of isometric force involves significant contributions due to cooperativity in cross-bridge binding (for most recent review see Gordon et al., 2000), i.e., NEM-S1 increased the Ca^{2+} sensitivity of force. The fact that partial extraction of TnC reduced the Ca^{2+} sensitivity of force suggests that cooperation in cross-bridge binding occurs both within and between neighboring functional groups.

Our results also indicate that the Ca^{2+} dependence of the kinetics of force development can be completely eliminated by a combination of strong-binding cross-bridges (treatment with NEM-S1) and disruption of near-neighbor cooperativity in the thin filament (partial extraction of TnC). Although it is tempting to conclude from these results that cross-bridge binding is the primary activator of cross-bridge kinetics, our results do not exclude the possibility that other mechanisms are operative under physiological conditions, where the number of strongly bound cross-bridges is certainly less than in our experiments with NEM-S1. In support of the this idea, k_{tr} in control fibers did not increase substantially until Ca^{2+}-activated isometric forces were greater than half-maximal (Fig. 7 A). Thus, it is possible in our experiments that strong binding cross-bridges in the form of NEM-S1 are such potent activators of the thin filament that other activating processes involving the effects of Ca^{2+} binding to potential regulatory sites, such as regulatory light chain (Diffee et al., 1995), are masked. Another possibility is that cross-bridge binding to the thin filament increases Ca^{2+} binding affinity of TnC, and the resulting increase in Ca^{2+} binding increases f_{app} (for review see Gordon et al., 2000).

We also observed that the effects on k_{tr} because of NEM-S1 alone were greater than the effects of TnC extraction alone. At first glance, this suggests that cooperativity in the activation of contraction is predominantly due to cross-bridge binding within functional groups, with much lesser contributions due to near-neighbor cooperation between adjacent functional groups. However, it must again be recognized that the addition of NEM-S1 in our experiments substantially increases the

number of strong-binding cross-bridges above that normally found in skeletal muscle fibers under physiological conditions. Thus, it is likely that strong-binding cross-bridges are not as dominant in activating contraction in living muscles, and that near-neighbor mechanisms play a proportionately greater role than implied by our present results with NEM-S1. Our finding that partial extraction of TnC was required to completely eliminate the activation dependence of k_{tr} is consistent with this conclusion.

REFERENCES

Brandt, P., and F. Schachat. 1997. Troponin C modulates the activation of the thin filaments by rigor cross-bridges. *Biophys. J.* 72: 2262–2267.

Brandt, P.W., M.S. Diamond, and F.H. Schachat. 1984. The thin filament of vertebrate skeletal muscle co-operatively activates as a unit. *J. Mol. Biol.* 180:379–384.

Brandt, P.W., D. Roemer, and F.H. Schachat. 1990. Cooperative activation of skeletal muscle thin filaments by rigor crossbridges: the effect of troponin C extraction. *J. Mol. Biol.* 212:473–480.

Brenner, B. 1988. Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: implications for regulation of muscle contraction. *Proc. Natl. Acad. Sci. USA.* 85:3265–3269.

Brenner, B., and E. Eisenberg. 1986. Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. *Proc. Natl. Acad. Sci. USA.* 83:3542–3546.

Brenner, B., and J.M. Chalovich. 1999. Kinetics of thin filament activation probed by fluorescence of N-((2-(iodoacetoxy)ethyl)-N-methyl)amino-7-nitrobenz-2-oxa-1,3-diazole-labeled troponin I incorporated into skinned fibers of rabbit psoas muscle: Implications for regulation of muscle contraction. *Biophys. J.* 77:2692–2708.

Campbell, K. 1997. Rate constant of muscle force redevelopment reflects cooperative activation as well as cross-bridge kinetics. *Biophys. J.* 72:254–262.

Campbell, K.S., and R.L. Moss. 2000. A thixotropic effect in contracting rabbit psoas muscle: prior movement reduces the initial tension response to stretch. *J. Physiol.* 525:531–548.

Diffee, G.M., M.L. Greaser, F.C. Reinach, and R.L. Moss. 1995. Effects of a non-divalent cation binding mutant of myosin regulatory light chain on tension generation in skinned skeletal muscle fibers. *Biophys. J.* 68:1443–1452.

Fabiato, A. 1988. Computer programs for calculating total from specified free and free from specified total ionic concentrations in aqueous solutions containing multiple metals or ligands. *Methods Enzymol.* 157:378–417.

Geeves, M.A., and S.S. Lehrer. 1994. Dynamics of the muscle thin filament regulatory switch: the size of the cooperative unit. *Biophys. J.* 67:273–282.

Godt, R.E., and B.D. Lindley. 1982. Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibers of the frog. *J. Gen. Physiol.* 80:279–297.

Gordon, A.M., E. Homsher, and M. Regnier. 2000. Regulation of

contraction in striated muscle. *Physiol. Rev.* 80:853–924.

Grabarek, Z., T. Tao, and J. Gergely. 1992. Molecular mechanism of troponin-C function. *J. Muscle Res. Cell Motil.* 13:383–393.

Greene, L.E., D.L. Williams, and E. Eisenberg. 1987. Regulation of actomyosin ATPase activity by troponin-tropomyosin: effect of the binding of the myosin subfragment 1 (S-1)-ATP complex. *Proc. Natl. Acad. Sci. USA.* 84:3102–3106.

Hancock, W.O., L.L. Huntsman, and A.M. Gordon. 1997. Models of calcium activation account for differences between skeletal and cardiac force redevelopment kinetics. *J. Muscle Res. Cell Motil.* 18:671–681.

Haselgrove, J.C. 1973. X-ray evidence for a conformational change in actin-containing filaments of vertebrate striated muscle. *Cold Spring Harbor Symp. Quant. Biol.* 37:225–234.

Huxley, H.E. 1973. Structural changes in actin- and myosin-containing filaments during contraction. *Cold Spring Harbor Symp. Quant. Biol.* 37:361–376.

Landesberg, A., and S. Sideman. 1994. Coupling calcium binding to troponin C and cross-bridge cycling in skinned cardiac cells. *Am. J. Physiol.* 266:H1260–H1271.

Lehrer, S.S. 1994. The regulatory switch of the muscle thin filament: Ca^{2+} or myosin heads? *J. Muscle Res. Cell Motil.* 15:232–236.

McKillop, D.F.A., and M.A. Geeves. 1993. Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament. *Biophys. J.* 65:693–701.

Metzger, J.M., and R.L. Moss. 1991. Kinetics of a Ca^{2+}-sensitive cross-bridge state transition in skeletal muscle fibers. *J. Gen. Physiol.* 98:233–248.

Metzger, J.M., M.L. Greaser, and R.L. Moss. 1989. Variations in cross-bridge attachment rate and tension with phosphorylation of myosin in mammalian skinned skeletal muscle fibers. *J. Gen. Physiol.* 93:855–883.

Moss, R.L. 1979. Sarcomere length-tension relations of frog skinned muscle fibers during calcium activation at short lengths. *J. Physiol.* 292:177–202.

Moss, R.L. 1992. Ca^{2+} regulation of mechanical properties of striated muscle. *Circ. Res.* 70:865–884.

Moss, R.L., G.G. Giulian, and M.L. Greaser. 1985. The effects of partial extraction of TnC upon the tension-pCa relationship in rabbit-skinned skeletal muscle fibers. *J. Gen. Physiol.* 86:585–600.

Palmer, S., and J.C. Kentish. 1998. Roles of Ca^{2+} and crossbridge kinetics in determining the maximum rates of Ca^{2+} activation and relaxation in rat and guinea pig skinned trabeculae. *Circ. Res.* 83:179–186.

Parry, D.A.D., and J.M. Squire. 1973. Structural role of tropomyosin in muscle regulation: analysis of the x-ray patterns from relaxed and contracting muscles. *J. Mol. Biol.* 75:33–55.

Razumova, M.V., A.E. Bukatina, and K.B. Campbell. 2000. Different myofilament nearest-neighbor interactions have distinctive effects on contractile behavior. *Biophys. J.* 78:3120–3137.

Regnier, M., D.A. Martyn, and P.B. Chase. 1998. Calcium regulation of tension redevelopment kinetics with 2-deoxy-ATP or low [ATP] in skinned rabbit psoas fibers. *Biophys. J.* 74:2005–2015.

Strang, K.T., N.K. Sweitzer, M.L. Greaser, and R.L. Moss. 1994. β-Adrenergic receptor stimulation increases unloaded shortening velocity of skinned single ventricular myocytes from rats. *Circ. Res.* 74:542–549.

Swartz, D.R., and R.L. Moss. 1992. Influence of a strong-binding myosin analogue on calcium-sensitive mechanical properties of skinned skeletal muscle fibers. *J. Biol. Chem.* 267:20497–20506.

Swartz, D.R., R.L. Moss, and M.L. Greaser. 1996. Calcium alone does not fully activate the thin filament for S1 binding to rigor myofibrils. *Biophys. J.* 71:1891–1904.

Sweitzer, N.K., and R.L. Moss. 1993. Determinants of loaded shortening velocity in single cardiac myocytes permeabilized with α-hemolysin. *Circ. Res.* 73:1150–1162.

Williams, D.L., L.E. Greene, and E. Eisenberg. 1984. Comparison of effects of smooth and skeletal muscle tropomyosins on interactions of actin and myosin subfragment 1. *Biochemistry.* 23:4150–4155.

Williams, D.L., L.E. Greene, and E. Eisenberg. 1988. Cooperative turning on of myosin subfragment 1 adenosine triphosphatase activity by the troponin-tropomyosin-actin complex. *Biochemistry.* 27:6987–6993.