Rate Constant of Muscle Force Redevelopment Reflects Cooperative Activation as Well as Cross-Bridge Kinetics

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ABSTRACT The rate of muscle force redevelopment after release-restretch protocols has previously been interpreted using a simple two-state cross-bridge cycling model with rate constants for transitions between non-force-bearing and force-bearing states, f, and between force-bearing and non-force-bearing states, g. Changes in the rate constant of force redevelopment, as with varying levels of Ca^{2+} activation, have traditionally been attributed to Ca^{2+}-dependent f. The current work adds to this original model a state of unactivated, noncycling cross-bridges. The resulting differential equation for activated, force-bearing cross-bridges, N_{cf}, was $\dot{N}_{cf} = -[g + f(K/(K + 1))]N_{cf} + f(K/(K + 1))N_T$, where K is an equilibrium constant defining the distribution between cycling and noncycling cross-bridges and N_T is the total number of cross-bridges. Cooperativity by which force-bearing cross-bridges participate in their own activation was introduced by making K depend on N_{cf}. Model results demonstrated that such cooperativity, which tends to enhance force generation at low levels of Ca^{2+} activation, has a counter-intuitive effect of slowing force redevelopment. These dynamic effects of cooperativity are most pronounced at low Ca^{2+} activation. As Ca^{2+} activation increases, the cooperative effects become less important to the dynamics of force redevelopment and, at the highest levels of Ca^{2+} activation, the dynamics of force redevelopment reflect factors other than cooperative mechanisms. These results expand on earlier interpretations of Ca^{2+} dependence of force redevelopment; rather than Ca^{2+}-dependent f, Ca^{2+}-dependent force redevelopment arises from changing expressions of cooperativity between force-bearing cross-bridges and activation.

INTRODUCTION

In constantly activated muscle, force redevelops with a characteristic rate constant after mechanical perturbation of muscle designed to break nearly all force-bearing cross-bridges and momentarily achieve zero force. This rate constant has been widely used to assess the sensitivity of cross-bridge kinetics to various factors, including the level of Ca^{2+} activation. The general idea has been that if a population of activated and cycling cross-bridges could be forced into a state in which no cross-bridges were generating force, then the time course over which the population reestablished force-generating capability would be a measure of the kinetics governing cyclic transitions between non-force-bearing and force-bearing states. Any dependence of the rate constant of force redevelopment on experimentally modified factors has been taken as an indication of a dependence of cross-bridge kinetics on the factor that was modified.

The rate constant of force redevelopment after mechanical disruption of force-bearing cross-bridges by release and restretch has been shown to be dependent on the level of Ca^{2+} activation in both skeletal (Brenner, 1988; Metzger and Moss, 1990) and cardiac (Wolff et al., 1995) muscle. In accord with these findings, the rate constant of force development after photorelease of Ca^{2+} from caged compounds in rabbit psoas and rat trabecular muscle has been shown to depend on the amount of Ca^{2+} released (Araujo and Walker, 1994). These results have been interpreted to indicate that the rate constant governing the formation of force-bearing from non-force-bearing cross-bridges, f, is Ca^{2+} dependent.

In this report, the original two-state interpretive model with cross-bridge kinetic constants f and g is extended to include an activation (recruitment) component. The activation process is influenced by cooperativity in which force-bearing cross-bridges act to enhance both activation and the formation of more force-bearing cross-bridges. It is shown that these cooperative actions actually slow the development of force and that this slowing is most pronounced at low levels of Ca^{2+} activation. There is less impact of cooperative actions on the time course of force development at high levels of Ca^{2+} activation. The net impact of these cooperative effects is to make the rate constant of force development Ca^{2+} dependent. In fact, the different rate constants at different levels of activation are due to different degrees of participation by cooperative mechanisms in the dynamics of force development.

MODELING

In accord with the original interpretive model for force redevelopment (Brenner, 1988), the simplest possible model of myofilament activation and cross-bridge cycling is used. The model on which the analysis is based (Fig. 1) is a reduced three-state version of a more complicated six-state model given in the Appendix and in Fig. 6. This six-state

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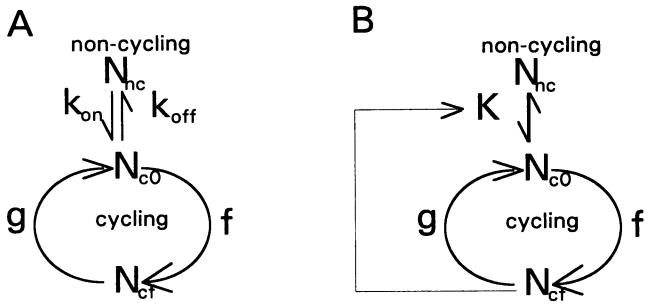

FIGURE 1 (*A*) Cross-bridge model depicting activation and cycling. The rate factors k_{on} and k_{off} govern the switching on and off of thin-filament regulatory proteins that allow noncycling cross-bridges, N_{nc}, to enter a cycling population. Within the cycling population, the constants f and g represent factors governing the transition from non-force-bearing states, N_{c0}, to force-bearing states, N_{cf}, and the transition from N_{cf} states back to N_{c0} states, respectively. (*B*) Entry into and exit from the cycling population is considered to be fast relative to cycling between non-force-bearing and force-bearing states. Thus k_{on} and k_{off} are replaced with the equilibrium activation factor K. Cooperativity between force-bearing states and activation is indicated by the feedback arrow between N_{cf} and K.

model merges some of the features of the model of T. L. Hill (1983) with some of the features of the activation model of Geeves and Lehrer (1994). It bears some similarity to several other models, including those of Yue (1987), Peterson et al. (1991), Landesberg and Sideman (1994), and Dobrunz et al. (1995). Our application is novel in that we analyze force redevelopment transients with a simplified three-state model that lumps several activation processes into a single state transition.

In the reduced model, the total cross-bridge population is divided into two subpopulations: a cycling population and a non-cycling population. Members of the cycling population can be found in one of two states: force-bearing, N_{cf}, and non-force-bearing, N_{c0}. The non-cycling population consists of a single non-force-bearing state, N_{nc}. Cross-bridges enter the cycling population from the non-cycling population according to a rate constant, k_{on}, and cross-bridges leave the cycling population according to a rate constant, k_{off}. It is shown in the Appendix that k_{on} and k_{off} incorporate both constants governing the switching on and off of thin filament regulatory proteins from steric blocking to nonblocking positions and constants governing the binding and dissociation of Ca^{2+} from the thin-filament regulatory protein. Once in the cycling population, cross-bridges undergo repeated transitions between force-bearing and non-force-bearing states, according to the rate constants f and g.

Activation

Activation is defined to be the result of processes that favor cross-bridge entry into the cycling population. This occurs when k_{on} increases relative to k_{off}. Thus Ca^{2+} causes activation by increasing the k_{on}/k_{off} ratio, resulting in a net movement of cross-bridges from the noncycling state, N_{nc}, into the cycling N_{c0} and N_{cf} states. In accord with data that show rapid movement of tropomyosin on thin filaments relative to S1 attachment and detachment (Geeves and Lehrer, 1994) and relatively rapid Ca^{2+} binding and dissociation, it is assumed that k_{on} and k_{off}, although they may be very different from one another, are both very much greater than f and g. Thus it may be considered that, at any time, an equilibrium exists in the exchange between N_{nc} and N_{c0} states relative to the cycling between N_{c0} and N_{cf} states. This equilibrium allows us to write

$$N_{c0} = \frac{k_{on}}{k_{off}} N_{nc} = KN_{nc}, \tag{1}$$

where K is an equilibrium constant equal to the ratio k_{on}/k_{off}. It is shown in the Appendix (Eq. A.12) that K is a saturatable function of Ca^{2+}; K is small, with few cross-bridges in the cycling population when Ca^{2+} is small, and K reaches a plateau with most of the cross-bridges in the cycling population when Ca^{2+} is high. For the purposes of this exercise, this saturatable relationship between K and Ca^{2+} will not be used but, instead, a simple linear relationship will suffice, such as

$$K = \alpha Ca^{2+}, \tag{2}$$

where α takes on a value appropriate to relations developed later. K will be termed an "activation factor."

Cross-bridge cycling

A differential equation expressing the rate of change of force-bearing cross-bridges may be written from inspection of Fig. 1 as

$$\dot{N}_{cf} = fN_{c0} - gN_{cf}, \tag{3}$$

where $\dot{N}_{cf}$ is the first time derivative of N_{cf}. It is shown in the Appendix how Eq. 3 applies, even when cycling cross-bridges may or may not have Ca^{2+} bound to TnC. To further develop the model, it is necessary to write this equation in terms of just N_{cf} and system constants.

With the total number of cross-bridges (cycling plus noncycling) fixed at a constant N_T, a conservation relationship among cross-bridges in the various states may be written as

$$N_T = N_{nc} + N_{c0} + N_{cf}. \tag{4}$$

Substituting Eqs. 1 and 4 into Eq. 3 and rearranging gives the desired differential equation in terms of just N_{cf} and system constants as

$$\dot{N}_{cf} = -\left[g + f\frac{K}{K+1}\right]N_{cf} + f\frac{K}{K+1}N_T. \tag{5}$$

The two terms on the right side of Eq. 5 represent two components causing dynamic change in N_{cf}: the first term causes N_{cf} to decrease at a rate proportional to N_{cf} itself, whereas the second term causes N_{cf} to increase at a rate proportional to N_T. This second term may be considered a

driving function. For fixed values of the factors g, f, and K, both the coefficient on N_{cf} in the first term on the right side and the driving function are constant. In this case, the rate of dynamic change in N_{cf} will be characterized entirely by the coefficient on N_{cf}, which will be called $k_{tr_{an}}$,

$$k_{tr_{an}} = g + f\frac{K}{K+1}, \tag{6}$$

where the "an" in the subscript identifies this rate constant as analytically derived, to distinguish it from an analogous rate constant to be determined later, which is estimated from the time course of force development.

For example, consider that N_{cf} has an initial value of zero, as is the case after a mechanical perturbation that breaks all force-bearing cross-bridges. Then, for constant values of g, f, and K in Eq. 5, N_{cf} will rise exponentially with time according to

$$N_{cf} = N_{ss}[1 - e^{-k_{tr_{an}}t}] \tag{7}$$

where N_{ss} is the asymptotic steady-state value of N_{cf} and is given by

$$N_{ss} = \frac{f(K/(K+1))}{k_{tr_{an}}} N_T. \tag{8}$$

During both steady state and the approach to steady state, force, F, is proportional to N_{cf}. Thus, by substituting F_{ss} in place of N_{ss} and F_T in place of N_T, Eqs. 5, 7, and 8 could be written in terms of F instead of N_{cf}. Furthermore, the rate constant of N_{cf} development, $k_{tr_{an}}$, is also the rate constant of force development. From Eq. 6 it is seen that $k_{tr_{an}}$ depends not only on the kinetic constants of cross-bridge cycling, f and g, but also on the activation factor K.

MODEL RESULTS

Ca^{2+} activation has a large affect on F_{ss} and a modest affect on $k_{tr_{an}}$

Because, by Eq. 2, K is a function of Ca^{2+}, the level of Ca^{2+} will determine both F_{ss} (according to Eq. 8) and $k_{tr_{an}}$ (according to Eq. 6). As Ca^{2+} varies from low to high, the factor $K/(K+1)$ in Eqs. 5, 6, and 8 varies between 0 and 1. Correspondingly, F_{ss} varies between 0 and F_{max} ($= [f/(f+g)]F_T$), and $k_{tr_{an}}$ varies between g and $f+g$. Low levels of Ca^{2+} result in $k_{tr_{an}}$ taking on values close to g such that force development is slower than at high levels of Ca^{2+}, where $k_{tr_{an}}$ takes on values closer to $f+g$. These predictions are borne out by graphical displays of Eq. 7 for several levels of Ca^{2+} activation in Fig. 2 ($f = g = 20\ s^{-1}$). A range of Ca^{2+} activation from $\alpha Ca^{2+} = 0.1$ to 1.5 was sufficient to change F from 16.7% to 75% of F_{max} (Fig. 2 *A*). The associated values of $k_{tr_{an}}$ ranged from 21.8 s^{-1} (just more than g) at the lowest level of Ca^{2+} activation to 32.0 s^{-1} (slightly more than midway between g and $f+g$) at the highest level. These differences in the time course of force redevelopment are shown clearly in Fig. 2 *B*. Therefore, a range of Ca^{2+} activation causing F to vary over most of its range has a modest effect on $k_{tr_{an}}$. These results are in accord with the findings of Landesberg and Sideman (1994), who found the rate constant of force development to increase with increasing activation in a four-state, loose-coupling skeletal muscle model with two low-affinity Ca^{2+}-binding sites and no cooperativity between force generation and Ca^{2+} binding to TnC.

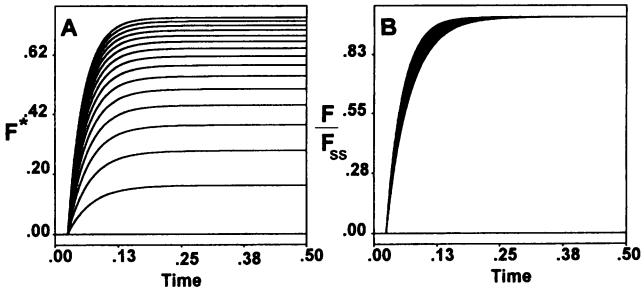

FIGURE 2 Time course of force development as predicted by Eq. 8 at different Ca^{2+} activation levels where the activation factor, $K = \alpha Ca^{2+}$, depends only on the level of Ca^{2+}. (*A*) Time course showing variation in both final steady-state force and rate of attainment of that steady-state force; highest steady-state force is obtained with the highest level of Ca^{2+} activation. Force, F^*, is expressed relative to the maximum force that would be developed at the highest possible activation. (*B*) Same data as in *A*, with force normalized to its steady-state value, F_{ss}, for each level of activation. This presentation emphasizes variation in rate of attainment of steady state with the different levels of Ca^{2+} activation; the curve with the slowest developing force is the one with the lowest level of Ca^{2+} activation, and the curve with the fastest developing force is the one with the highest level of Ca^{2+}activation.

Cooperativity between force-bearing cross-bridges and activation profoundly affects both F_{ss} and k_{tr}

Cooperativity between force-bearing cross-bridges and activation is introduced into the model by letting K depend not only on Ca^{2+} but also on N_{cf} according to a simple expansion of Eq. 2:

$$K = \alpha Ca^{2+}(1 + \beta N_{cf}) \tag{9}$$

where β is a coefficient defining the strength of the cooperativity. Cooperativity comes about in Eq. 9 because N_{cf} acts to enhance its own formation by increasing the activation factor K, which then recruits more cross-bridges into the cycling population from the noncycling population (this is indicated diagramatically by the feedback arrow in Fig. 1 *B*). This very general and nonspecific formulation of cooperative effects embraces a variety of cooperative mechanisms, including both enhanced Ca^{2+} binding to troponin C (Bremel and Weber, 1972; Hoffman and Fuchs, 1987; Wang and Fuchs, 1994) and enhanced switching on of the thin-filament regulatory unit with increased force-bearing cross-bridges (Geeves and Lehrer, 1994; Lehrer, 1994). Other forms of cooperativity, such as Ca^{2+}-enhanced Ca^{2+} binding and end-to-end interactions between adjacent reg-

ulatory units on the thin filament (Hill, 1983; Dobrunz et al., 1995), are probably not well represented by Eq. 9.

The cooperativity in Eq. 9 introduces nonlinearity into Eq. 5 because $K/(K + 1)$ becomes a function of N_{cf} for a fixed Ca^{2+}. This dependence of $K/(K + 1)$ on N_{cf} affects Eq. 7 by two mechanisms: 1) as N_{cf} increases, $k_{tr_{an}}$ apparently increases, and 2) as N_{cf} increases, the driving function, i.e., the second term on the right side of Eq. 5, also increases. At first glance, these effects would be anticipated to speed the rate of force redevelopment.

However, results from numerical solutions of the nonlinear Eq. 5 (Runge-Kutta integration; integration step size 0.0005 s; initial conditions $N_{cf} = 0; f = g = 20\ s^{-1}$), with cooperativity incorporated into K according to Eq. 9, produced results opposite those that were anticipated. These results were obtained for two situations: 1) a single degree of cooperativity ($\beta = 40$) and varying levels of Ca^{2+} activation to produce varying steady-state forces (Fig. 3); and 2) varying degrees of cooperativity ($\beta = 0, 10, 20, 30, 40, 50$) with corresponding varying levels of Ca^{2+} activation to produce the same steady-state force with each degree of cooperativity ($F_{ss} = 0.2F_{max}$) (Fig. 4). In the data of Fig. 4, the rate constant is not analytically derived but empirically determined by the inverse of the time required to reach 0.6321 of the final steady-state value. (Although, strictly speaking in this nonlinear formulation, the time course of rise in force is not purely monoexponential; its appearance is such that it could easily be mistaken as monoexponential. A variety of time courses of rise in force can be achieved by choosing different functional forms for cooperativity. However, the point to be made here is not the specific functional form for cooperativity, but to demonstrate that cooperative feedback between force-bearing cross-bridges and activation has a profound effect on k_{tr}, and this effect varies, depending on the level of Ca^{2+} activation.) This empirically determined rate constant will be termed k_{tr}. In Fig. 3, where steady-state force ranges from 10% of F_{max} at low activation ($\alpha Ca^{2+} = 0.025$) to 90% of F_{max} at high activation ($\alpha Ca^{2+} = 0.375$), k_{tr} ranged from 6.06 s^{-1} ($\ll g$) at the lowest level of activation to 27.8 s^{-1} (between g and $f + g$) at the highest level (Fig. 3 *A*).

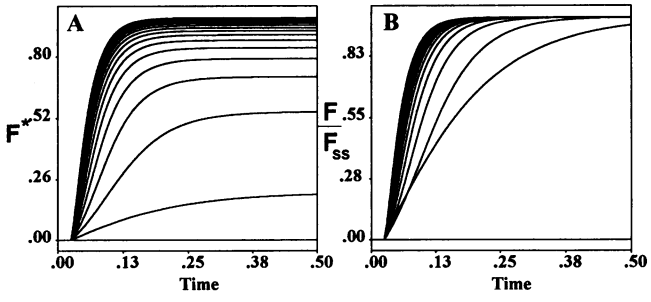

FIGURE 3 Time course of force development at different levels of activation as predicted by the model with cooperative feedback between force-bearing cross-bridges and activation, $K = \alpha Ca^{2+}(1 + \beta N_{cf})$. (*A*) Time course showing variation in both final steady-state force and rate of attainment of that steady-state force; highest steady-state force is obtained with the highest level of Ca^{2+} activation. Force, F^*, is expressed relative to the maximum force that would be developed at the highest possible activation. (*B*) Same data as in *A*, with force normalized to its steady-state value, F_{ss}, for each level of activation to emphasize the variation in rate of attainment of the steady state with the different levels of Ca^{2+} activation. The curve with the slowest developing force is the one at the lowest level of Ca^{2+} activation, and the curve with the fastest developing force is the one at the highest level of Ca^{2+} activation.

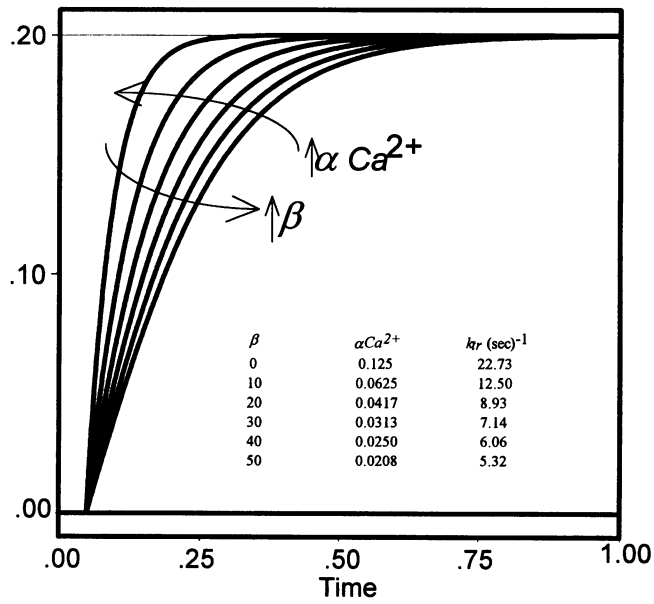

FIGURE 4 Varying degrees of cooperativity ($\beta = 0, 10, 20, 30, 40, 50$) and corresponding varying levels of Ca^{2+} activation to produce in each case the same steady-state force ($F_{ss} = 0.2F_{max}$). k_{tr} and the time course of force development decrease as cooperativity increases (see tabular inset).

It is clearly evident from these results and from the display in Fig. 3 *B* that force development at low levels of Ca^{2+} activation is much slower than force development at high levels. Furthermore, in comparing the time courses of force redevelopment in Fig. 3 (where cooperativity was operative) with those in Fig. 2 (where it was not), it is apparent that the rates of force development at the highest levels of activation are not so different ($k_{tr_{an}} = 32.0\ s^{-1}$ in Fig. 2 versus $k_{tr} = 27.8\ s^{-1}$ in Fig. 3), whereas the rates of force development at the lowest level of activation are very different ($k_{tr_{an}} = 21.8\ s^{-1}$ in Fig. 2 versus $k_{tr} = 6.0\ s^{-1}$ in Fig. 3). These relations between cooperativity, Ca^{2+} activation, and time course of force development are further shown in Fig. 4. As cooperativity increases (i.e., as β gets larger), not only does the amount of Ca^{2+} activation required to achieve the same steady force decrease, but k_{tr} also decreases, commensurate with the slowing of the time course of force development. Contrary to the situation in which cooperativity was absent and $k_{tr_{an}}$ values were bounded between g and $f + g$, the inclusion of cooperativity brings about a k_{tr} value considerably less than g at the lowest levels of activation. This surprising result is explained in the Discussion and by a linearized analysis in the Appendix.

From the results presented in Figs. 3 and 4, it can be concluded that the cooperative dependence of K on N_{cf} has a profound effect on the time course of force development. This effect is most pronounced when αCa^{2+} and N_{cf} are low enough that $K/(K + 1) \ll 1$. If αCa^{2+} and N_{cf} are sufficiently high, then $K/(K + 1)$ approaches 1 and any dependence of K on N_{cf} is of little consequence.

As seen above, the same cooperative actions that slow force development also act to increase steady-state force. Again, this effect is most pronounced at low levels of Ca^{2+} activation. The net result of these steady-state effects is that cooperativity causes a pronounced shift to the left of the force-log αCa^{2+} curve, as shown in Fig. 5 (obtained by solution of Eq. 8 for various values of αCa^{2+}). (Note that just as the activation factor K participates in the time course of force redevelopment, the cycling rate constants f and g participate in steady-state values of force according to Eq. 8, and consequently, these cycling parameters help to determine the location and shape of the force-log Ca^{2+} curve.) Note, in Fig. 5 *B*, that when the model predictions are plotted with $\log[F^*/(1 - F^*)]$ on the ordinate (where F^* is F/F_{max} and a value of $\log[F^*/(1 - F^*)] = 0.0$ on the ordinate represents $F^* = 0.5$), there is a steeper slope for negative than for positive values of $\log[F^*/(1 - F^*)]$. This agrees with experimental data reported for cardiac muscle by several authors (McDonald et al., 1995; Sweitzer and Moss, 1990; Gao et al., 1996) and reflects a greater influence of cooperative mechanisms at low Ca^{2+} than at high Ca^{2+}. Note also that when there is no cooperative feedback, there is a single slope over both negative and positive values of $\log[F^*/(1 - F^*)]$. Cooperative feedback actions responsible for shifting the force-log Ca^{2+} curve to the left (Fig. 5 *A*) and responsible for the steeper slope for negative values of $\log[F^*/(1 - F^*)]$ (Fig. 5 *B*) are the same actions that slow the time course of force development at low Ca^{2+} in Figs. 3 and 4.

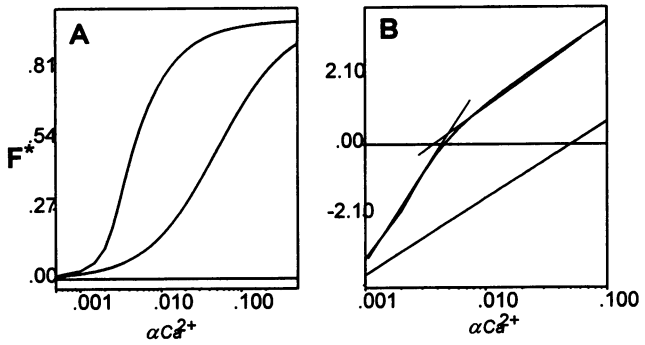

FIGURE 5 (*A*) Force-log Ca^{2+} curves for cases with (*left curve*) and without (*right curve*) cooperative feedback. (*B*) Same data as in *A*, but plotted with $\log[F^*/(1 - F^*)]$ on the ordinate. Value 0.0 on the ordinate represents $F^* = 0.5$. Steeper slope for negative than for positive values of $\log[F^*/(1 - F^*)]$ reflect greater influence of cooperative mechanisms at low Ca^{2+}. Note single slope for predictions from model without cooperative feedback. Cooperative feedback actions responsible for shifting the force-log Ca^{2+} curve to the left are the same actions that slow the time course of force development at low Ca^{2+} in Figs. 3 and 4.

DISCUSSION

Cooperativity slows force development in a Ca^{2+}-dependent manner

In a recent review, Solaro and Van Eyk (1996) asked, "What is the relative role of feedback effects of cross-bridge binding on thin filament activity?" They answered that question by assessing the role of such positive feedback in the length-dependent activation of cardiac muscle. In the current study we add a second important aspect to this positive feedback, namely, feedback between force-bearing cross-bridges and activation, whereas enhancing steady-state force slows the rate of force development. This cooperation-induced slowing is most pronounced when Ca^{2+} activation is low and becomes less obvious when Ca^{2+} activation is high. The net impact is to make the rate constant of force development Ca^{2+} dependent. This outcome of our modeling studies was presaged by at least four earlier studies. T. L. Hill (1983), in his equilibrium and steady-state analysis of four-state activation and cross-bridge cycling models, demonstrated that if Ca^{2+} binds more strongly to troponin when myosin is attached, then the effective consequence in a two-state cross-bridge model would be to increase the rate constant of myosin attachment to actin. Effectively, this would, using our Eq. 6, increase k_{tr}. Thus, had Hill performed studies of mechanical transients with his models, he would have predicted that enhanced Ca^{2+} binding to troponin with cross-bridge binding to actin would change k_{tr} in a manner predicted by our model. In an experimental study, Millar and Homsher (1990) argued that the fact that k_{tr} was sensitive to Ca^{2+} in contrast to the fact that the rate constant characterizing the tension transient after phosphate release was not sensitive to Ca^{2+} could be explained on the basis of a model in which strong cross-bridges activate the thin filament. Our model essentially embodies this argument. In another experimental study, Swartz and Moss (1992) used NEM-S1 subfragments in skinned skeletal muscle fibers and found them to enhance force and increase k_{tr} at low Ca^{2+}, two observations that are consistent with our modeling results. Finally, as already noted in the previously cited modeling study by Landesberg and Sideman (1994), the predictions that there would be lower k_{tr} at low force than at high force is completely consistent with our findings. In the current study we go beyond these earlier works to explicitly elucidate the dynamic consequences of cooperativity using the simplest possible model.

An intuitive appreciation of cooperative-induced slowing of force development is difficult to develop. One approach is to consider force development as an incremental process and apply the results of a linearized form of the nonlinear equation at different increments of behavior as is done in the Appendix. Another is as follows. Consider force development at low levels of Ca^{2+} activation when cooperativity is most strongly expressed. From Eq. 8, it can be deduced that, in the presence of cooperativity, the steady-state level to

which N_{cf} will eventually rise in a force development episode is a function of N_{cf} such that as N_{cf} rises, the steady-state level also rises. In a sense, cooperative feedback causes N_{cf} to chase after an ever rising final value. Eventually, N_{cf} catches up with its increasing steady-state value and the force development process terminates. However, the time required for N_{cf} to catch up with its rising steady-state value causes force development to take longer than would have been the case had the steady-state value remained constant and not been progressively moving ahead. The net effect is to generate an apparently slow developing process with a low-value k_{tr}.

Contrast the time course at low levels of Ca^{2+} activation with the time course at high levels of activation. It is seen in the Model Results and argued in the Appendix that at high levels of activation, the dependence of the steady-state value on N_{cf} as a result of cooperative feedback is not nearly as strong as at low levels of Ca^{2+} activation. Thus as N_{cf} increases, the steady-state value does not move away to higher values. This makes it possible to reach the steady-state value in a shorter period of time. The net result is that force development is completed more quickly and k_{tr} is greater than at low levels of Ca^{2+} activation.

Differences in Ca^{2+} dependence of k_{tr} between cardiac and skeletal muscle is largely a matter of greater cooperativity in skeletal muscle

Determined experimentally in fast skeletal muscle, k_{tr} has been shown to vary with Ca^{2+} over a broad range from fivefold (Brenner, 1988) to more than 10 fold (Metzger and Moss, 1990). In contrast, k_{tr} in cardiac muscle is much less sensitive to Ca^{2+}, with reports varying from a threefold variation (Wolff et al., 1995) to no variation at all (Hancock et al., 1993). To account for differences between skeletal and cardiac muscle with the model, we need only postulate that cooperativity is greater in skeletal muscle than in cardiac muscle. As we have shown, over less than a full range of activation, no cooperativity (i.e., $\beta = 0$) resulted in less than 1.5-fold dependence of k_{tr} on the level of Ca^{2+} activation (Fig. 2), whereas greater amounts of cooperativity ($\beta = 40$) resulted in a threefold change in k_{tr} (Fig. 3). More cooperativity in the model ($\beta > 40$) would have generated an even wider range of k_{tr}. Another way to view these features of the model is that the highest value of k_{tr} is set by f and g, and the lowest value is set by β. In this way, one model with different parameters could accommodate a wide range of k_{tr} and of variations in k_{tr} with Ca^{2+} among different muscles.

Thus a postulate of the model is that there is a greater range of k_{tr} in skeletal than in cardiac muscle because there is more cooperativity in skeletal muscle activation than in cardiac muscle activation. Greater cooperativity in skeletal muscle activation is seen by the much higher Hill coefficients of the force-pCa curves in skeletal than in cardiac muscle (Morimoto and Ohtsuki, 1994) and by other evidence (see review by Moss, 1992). As explained by Moss (1992) and as shown in this modeling exercise, this cooperativity has its greatest effect at low Ca^{2+} activation. The result is that at low Ca^{2+} activation, the k_{tr} of fast skeletal muscle, with presumably relatively high f and g, is of the same value ($<5\ s^{-1}$) as is commonly found in cardiac muscle, presumably with slower f and g.

Nonlinearities from cooperative feedback introduce dependence of force development on initial conditions

There is an additional consequence of the nonlinearities from cooperative feedback. This is that, for a given level of Ca^{2+} activation, the initial conditions from which force is redeveloping will have a profound effect on k_{tr} (these initial condition effects are strictly a feature of the nonlinear character of cooperative feedback and would not appear in a linear system). Thus different conclusions may be drawn from experiments that are done differently such that they generate different initial conditions just before force redevelopment. For instance, the discrepancy between the findings of Hancock et al. (1993) and Wolff et al. (1995) with regard to Ca^{2+} effects on force redevelopment in cardiac muscle could very well be due to different initial conditions at the onset of the force redevelopment period in these two experiments. Differences in protocols may have had very different effects on breaking of cross-bridges, and thus there may have been markedly different initial conditions. Protocols that do not cause all cross-bridges to detach will reduce the cooperative effect on the rate constant, with the result that the Ca^{2+} dependence of the rate constant of force redevelopment would become much less apparent.

Cooperativity introduces ambiguity in the relation between k_{tr} and f

Finally, it should be noted that these cooperative effects appear in Eq. 6 as changes in a multiplier of the cross-bridge cycling rate constant f such that if these cooperative effects were not considered, they would be mistaken as changes in f itself. Thus the results from force redevelopment experiments are ambiguous; changes in the rate constant of force redevelopment may represent either a change in cross-bridge kinetics or varying expressions of cooperative interactions between force-bearing cross-bridges and activation.

CONCLUSION

In conclusion, cooperative interactions between force-bearing cross-bridges and activation can have a profound affect on contractile system dynamics. Most importantly, the time course of force redevelopment is strongly affected by this cooperativity and does not necessarily represent cross-bridge kinetics alone. Furthermore, these cooperative effects are exhibited to varying degrees, depending on the

level of Ca^{2+} activation; they are most pronounced at low levels of Ca^{2+} activation. Finally, these cooperative effects act to slow down the contractile system response, with the consequence of making an inherently fast cross-bridge cycling system appear to be much slower than one would expect.

APPENDIX

Simple activation and cross-bridge-cycle model from a more complex Ca^{2+}-binding, activation model

Consider the six-state myofilament activation and cross-bridge cycling model in Fig. 6. A thin-filament, myosin-binding site is represented by the chain of three circles (no stoichiometric relations are implied by the three rings). The regulatory Tm-Tn unit governing myosin head access to the thin-filament binding site is represented by the bar spanning the binding site. The myosin head is represented by the triangle. The regulatory unit may be in one of two steric configurations: the "off" position (states N_1 and N_2) or the "on" position (states N_3, N_4, N_5, and N_6). Switching between the "off" and "on" positions is governed by the on rate constants k', k'_{on}, and the off constant, k_{off}. Myosin heads can attach (strongly bind) to the thin filament only when the regulatory unit is in the "on" postition. Attachment (strong binding) and detachment (weak binding) occur cyclically according to rate constants f and g. Force is generated when myosin heads are attached (states N_5 and N_6). Ca^{2+} binds and dissociates from the low-affinity TnC site on the regulatory unit with rate constants of association, k^+, and dissociation, k_-. When Ca^{2+} is not bound to TnC and the regulatory unit is "off" (state N_1), there is very little probability that the "off" regulatory unit will turn "on" (i.e., $k' \ll k_{off}$). When Ca^{2+} is bound to TnC and the regulatory unit is "off" (state N_2), there is a much greater probability that the "off" regulatory unit will turn "on" (i.e., $k_{on} > k_{off}$). Ca^{2+} may bind and dissociate from TnC, regardless of whether the regulatory unit is "on" or "off" and whether myosin heads are attached or detached. When myosin heads are attached in the force-bearing states (N_5 and N_6), there is no probablity that the regulatory unit will turn "off," and it must await myosin head detachment before doing so. N_6 is a force-bearing state that does not have Ca^{2+} bound to TnC. In the absence of available Ca^{2+}, N_6 persists until cross-brides detach to form N_4. Once in N_4, there is a finite probability that myosin heads will reattach to once again form N_6, but the chance of this is small, because $k_{off} \gg f$, and the greatest probability is that N_4 will progress to N_1.

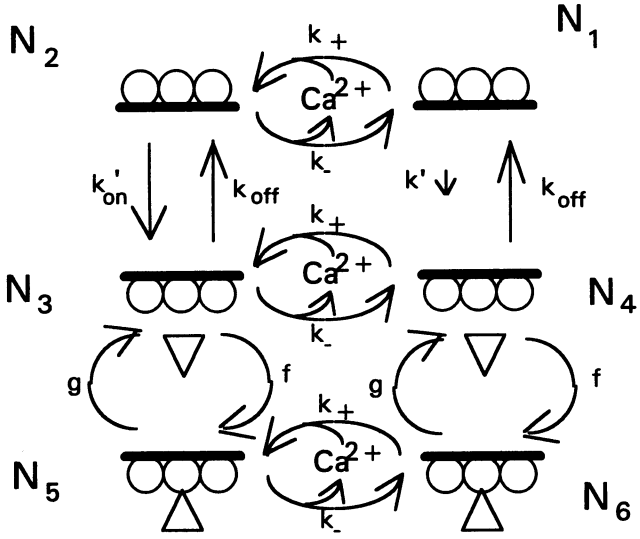

FIGURE 6 Six-state cross-bridge activation and cycling model. See text for definitions and explanations.

Differential equations describing the rate of change of all states may be written from inspection of Fig. 6 as follows:

$$\dot{N}_1 = k_{off}N_4 + k_-N_2 - [k_+Ca^{2+} + k']N_1 \quad (A.1)$$

$$\dot{N}_2 = k_+Ca^{2+}N_1 + k_{off}N_3 - [k'_{on+k_-}]N_2 \quad (A.2)$$

$$\dot{N}_3 = k'_{on}N_2 + k_+Ca^{2+}N_4 + gN_5 - [k_- + k_{off} + f]N_3 \quad (A.3)$$

$$\dot{N}_4 = k'N_1 + k_-N_3 + gN_6 - [k_+Ca^{2+} + k_{off} + f]N_4 \quad (A.4)$$

$$\dot{N}_5 = fN_3 + k_+Ca^{2+}N_6 - [k_- + g]N_5 \quad (A.5)$$

$$\dot{N}_6 = fN_4 + k_-N_5 - [k_+Ca^{2+} + g]N_6. \quad (A.6)$$

A reduced set of equations may be formed by combining the various states as follows. Combine the two "off" states (N_1 and N_2) into a single noncycling state, N_{nc}; combine the two cycling but non-force-bearing states (N_3 and N_4) into a single state, N_{c0}; combine the two force-bearing states (N_5 and N_6) into a single force-bearing state, N_{cf}. This makes it possible to rewrite Eqs. A.1–A.6 as three equations:

$$\dot{N}_{nc} = k_{off}N_{c0} - k_{on}N_{nc} \quad (A.7)$$

$$\dot{N}_{c0} = [gN_{cf} - fN_{c0}] + [k_{on}N_{nc} - k_{off}N_{c0}] \quad (A.8)$$

$$\dot{N}_{cf} = fN_{c0} - gN_{cf}, \quad (A.9)$$

where

$$k_{on} = \left[k' + (k'_{on} - k')\frac{N_2}{N_1 + N_2}\right] = k'_{on}\frac{N_2}{N_1 + N_2}; \quad \text{if } k' = 0. \quad (A.10)$$

In the reduced formulation (A.7–A.9), Ca^{2+} appears only implicitly as a determinant of the coefficient k_{on} through its effect on the ratio $N_2/(N_2 + N_1)$. To an approximation,

$$\frac{N_2}{N_1 + N_2} = \frac{Ca^{2+}}{(k_-/k_+) + Ca^{2+}} \quad (A.11)$$

where $k_-/k_+ = Ca_{1/2}$ and is the concentration of Ca^{2+} at which the ratio equals 0.5. At high Ca^{2+} that ratio saturates at 1; at low Ca^{2+} it approaches 0.

Further model reduction is achieved by employing the assumption made in the text that k_{on} and k_{off} are large relative to f and g. Under these conditions and in the time frame in which changes in force-bearing cross-bridges occur, $\dot{N}_{nc} \approx 0$, and an equilibrium constant, K, between N_{nc} and N_{c0} may be defined as

$$K = \frac{k_{on}}{k_{off}} = \frac{k'_{on}}{k_{off}}\left[\frac{Ca^{2+}}{(k_-/k_+) + Ca^{2+}}\right]. \quad (A.12)$$

Equation A.12 gives an explicit dependence of K on 1) Ca^{2+}; 2) the "on"–"off" switching constants; and 3) the Ca^{2+} association and dissociation constants. It is a more specific form of the very general Eq. 1 in the text. With the definition of K, the reduced set of differential equations (A.7–A.9) may be further reduced to the single differential equation given in the text as Eq. 5.

Various forms of cooperativity may be represented phenomenologically by a single equation

Cooperativity in which force-bearing cross-bridges facilitate their own formation may come about by any of several mechanisms. For our purposes, we consider only two possibilities: 1) force-bearing cross-bridges enhance the switching "on" coefficients (k_{on}' and/or k') relative to the switching "off" coefficient, and 2) force-bearing cross-bridges enhance the Ca^{2+} binding constant (k_+) relative to the Ca^{2+} dissociation constant (k_-), i.e., they decrease $Ca_{1/2}$. For instance, a cooperativity by which force-bearing cross-bridges enhance switching "on" may be represented by

$$\frac{k_{on}'}{k_{off}} = \alpha_1(1 + \beta_1 N_{cf}),$$

where α_1 is the zero N_{cf} switching constant and β_1 is a cooperativity coefficient. Similarly, cooperativity by which force-bearing cross-bridges enhance Ca^{2+} binding may be represented by

$$\frac{k_+}{k_-} = \alpha_2(1 + \beta_2 N_{cf}),$$

where α_2 is the zero N_{cf} Ca^{2+}-binding constant and β_2 is a cooperativity coefficient. Substituting either form of cooperativity into Eq. A.12 and approximating the result can result in an equation of the form of Eq. 9 in the text. Thus Eq. 9 makes no implication as to the mechanism of cooperativity and may represent many cooperative mechanisms.

Linear approximation for interpretation of effect of cooperativity on k_{tr}

Cooperativity-induced slowing in force development may be appreciated by analyzing a linear approximation to the nonlinear Eq. 5 created by the inclusion of the cooperative relation in Eq. 9. Consider behavior around some value of N_{cf} equal to N_0. Then the dependence of the factor $K/(K + 1)$ on N_{cf} may be linearly approximated by a truncated Taylor expansion in which second-order and higher terms have been dropped:

$$\frac{K}{K+1} \approx K_0 + K_1 N_{cf}, \qquad (A.13)$$

where K_0 and K_1 are constants. K_1 is the partial derivative of $K/(K + 1)$ with respect to N_{cf} evaluated at $N_{cf} = N_0$ and can be shown to be

$$K_1 = \frac{\alpha Ca^{2+}\beta}{[1 + \alpha Ca^{2+}(1 + \beta N_0)]^2}. \qquad (A.14)$$

Substituting Eq. A.14 into Eq. A.13 allows us to calculate K_0 as

$$K_0 = \frac{\alpha Ca^{2+}}{1 + \alpha Ca^{2+}(1 + \beta N_0)}\left\{1 + \frac{\alpha Ca^{2+}(1 + \beta N_0)\beta N_0}{1 + \alpha Ca^{2+}(1 + \beta N_0)}\right\}. \qquad (A.15)$$

Both K_0 and K_1 depend on αCa^{2+}, the cooperative factor β, and the value chosen for N_0. However, K_1 has a stronger dependence on β than does K_0, such that strong cooperativity (i.e., high β) tends to increase K_1 more than K_0. Furthermore, at low Ca^{2+} and low N_0, K_1 tends to dominate over K_0, making $K/(K + 1) \approx K_0 + K_1 N_{cf}$ more strongly dependent on N_{cf} than it would be at higher Ca^{2+}.

Substituting the approximation of $K/(K + 1)$ given in Eq. A.13 into Eq. 5 makes it possible to rewrite the differential equation as

$$\dot{N}_{cf} = -[g + f(K_0 - AK_1)]N_{cf} - fK_1N_{cf}^2 + fK_0N_T. \qquad (A.16)$$

Linearization of Eq. A.16 around $N_{cf} = N_0$ and expressing in terms of incremental changes in N_{cf} around N_0, which we will call η, gives

$$\dot{\eta} = -[g + f(K_0(1 + 2N_0) - K_1N_T)]\eta + \delta, \qquad (A.17)$$

where δ is some incremental part of fK_0A. In this linearized equation, an incremental k_{tr} may be defined as

$$k_{tr_{inc}} = g + f(K_0(1 + 2N_0) - K_1N_T). \qquad (A.18)$$

Values of $k_{tr_{inc}}$ vary according to N_0 and the relative values of K_0 and K_1 as follows:

$$g < k_{tr_{inc}} < g + f$$

if

$$0 < K_0(1 + 2N_0) - K_1N_T < 1 \qquad (A.19)$$

$$k_{tr_{inc}} < g$$

if

$$K_1N_T > K_0(1 + 2N_0).$$

Both ranges are possible. At low Ca^{2+} activation (low N_0) and strong cooperativity (high β), it can be shown that K_1N_T is relatively greater than $K_0(1 + 2N_0)$, with the result that $k_{tr_{inc}}$ is even less than g. Such a low value of $k_{tr_{inc}}$ means that force develops more slowly than one would expect from even the slowest step in the cross-bridge cycle. At high levels of Ca^{2+} activation, $K_0(1 + 2N_0)$ begins to approach K_1N_T and $k_{tr_{inc}}$ falls in the range between g and $f + g$. In this linear approximation there is nothing to stop cooperative feedback from slowing the system response to the point where it is infinitesimally slow and then, with stronger cooperative effects, to cause the system to become unstable. The nonlinear features of the original equation limit this destabilizing effect and prevent instability. The surprising result from this analysis is that positive feedback from cooperative effects may make an inherently fast cross-bridge cycling system appear to be slow.

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