

# 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  $\text{Ca}^{2+}$  activation, have traditionally been attributed to  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  activation, has a counter-intuitive effect of slowing force redevelopment. These dynamic effects of cooperativity are most pronounced at low  $\text{Ca}^{2+}$  activation. As  $\text{Ca}^{2+}$  activation increases, the cooperative effects become less important to the dynamics of force redevelopment and, at the highest levels of  $\text{Ca}^{2+}$  activation, the dynamics of force redevelopment reflect factors other than cooperative mechanisms. These results expand on earlier interpretations of  $\text{Ca}^{2+}$  dependence of force redevelopment; rather than  $\text{Ca}^{2+}$ -dependent  $f$ ,  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  from caged compounds

in rabbit psoas and rat trabecular muscle has been shown to depend on the amount of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  activation. There is less impact of cooperative actions on the time course of force development at high levels of  $\text{Ca}^{2+}$  activation. The net impact of these cooperative effects is to make the rate constant of force development  $\text{Ca}^{2+}$  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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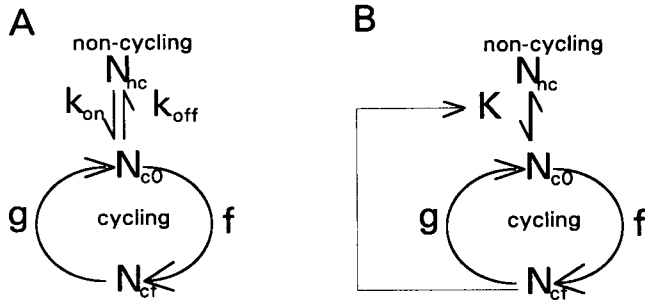


FIGURE 1 (A) Cross-bridge model depicting activation and cycling. The rate factors  $k_{on}$  and  $k_{off}$  govern the switching on and off of thin-filament regulatory proteins that allow noncycling cross-bridges,  $N_{nc}$ , to enter a cycling population. Within the cycling population, the constants  $f$  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

$$N_{c0} = \frac{k_{on}}{k_{off}} N_{nc} = K N_{nc}, \quad (1)$$

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

$$K = \alpha Ca^{2+}, \quad (2)$$

where  $\alpha$  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

$$\dot{N}_{cf} = f N_{c0} - g N_{cf}, \quad (3)$$

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

$$N_T = N_{nc} + N_{c0} + N_{cf}. \quad (4)$$

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

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

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  $k_{tran}$ ,

$$k_{tran} = g + f \frac{K}{K + 1}, \quad (6)$$

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

$$N_{cf} = N_{ss}[1 - e^{-k_{tran}t}] \quad (7)$$

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

$$N_{ss} = \frac{f(K/(K + 1))}{k_{tran}} N_T. \quad (8)$$

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,  $k_{tran}$ , is also the rate constant of force development. From Eq. 6 it is seen that  $k_{tran}$  depends not only on the kinetic constants of cross-bridge cycling,  $f$  and  $g$ , but also on the activation factor  $K$ .

## MODEL RESULTS

### $\text{Ca}^{2+}$ activation has a large effect on $F_{ss}$ and a modest effect on $k_{tran}$

Because, by Eq. 2,  $K$  is a function of  $\text{Ca}^{2+}$ , the level of  $\text{Ca}^{2+}$  will determine both  $F_{ss}$  (according to Eq. 8) and  $k_{tran}$  (according to Eq. 6). As  $\text{Ca}^{2+}$  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  $k_{tran}$  varies between  $g$  and  $f + g$ . Low levels of  $\text{Ca}^{2+}$  result in  $k_{tran}$  taking on values close to  $g$  such that force development is slower than at high levels of  $\text{Ca}^{2+}$ , where  $k_{tran}$  takes on values closer to  $f + g$ . These predictions are borne out by graphical displays of Eq. 7 for several levels of  $\text{Ca}^{2+}$  activation in Fig. 2 ( $f = g = 20 \text{ s}^{-1}$ ). A range of  $\text{Ca}^{2+}$  activation from  $\alpha\text{Ca}^{2+} = 0.1$  to 1.5 was sufficient to change  $F$  from 16.7% to 75% of  $F_{max}$  (Fig. 2 A). The associated values of  $k_{tran}$  ranged from  $21.8 \text{ s}^{-1}$  (just more than  $g$ ) at the lowest level of  $\text{Ca}^{2+}$  activation to  $32.0 \text{ s}^{-1}$  (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  $\text{Ca}^{2+}$

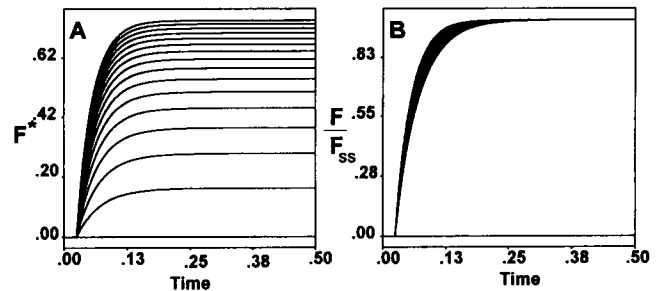


FIGURE 2 Time course of force development as predicted by Eq. 8 at different  $\text{Ca}^{2+}$  activation levels where the activation factor,  $K = \alpha\text{Ca}^{2+}$ , depends only on the level of  $\text{Ca}^{2+}$ . (A) Time course showing variation in both final steady-state force and rate of attainment of that steady-state force; highest steady-state force is obtained with the highest level of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  activation; the curve with the slowest developing force is the one with the lowest level of  $\text{Ca}^{2+}$  activation, and the curve with the fastest developing force is the one with the highest level of  $\text{Ca}^{2+}$  activation.

activation causing  $F$  to vary over most of its range has a modest effect on  $k_{tran}$ . 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  $\text{Ca}^{2+}$ -binding sites and no cooperativity between force generation and  $\text{Ca}^{2+}$  binding to TnC.

### 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  $\text{Ca}^{2+}$  but also on  $N_{cf}$  according to a simple expansion of Eq. 2:

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

where  $\beta$  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 diagrammatically 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -enhanced  $\text{Ca}^{2+}$  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  $N_{cf} = 0$ ;  $f = g = 20 \text{ s}^{-1}$ ), 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,

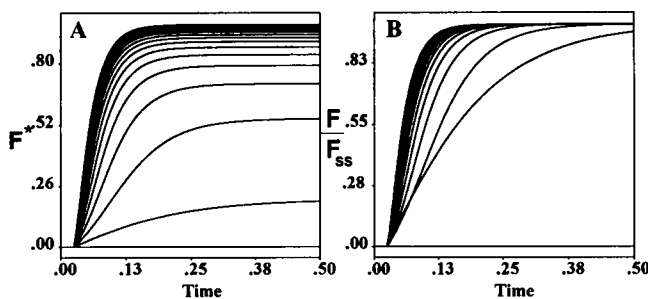


FIGURE 3 Time course of force development at different levels of activation as predicted by the model with cooperative feedback between force-bearing cross-bridges and activation,  $K = \alpha Ca^{2+} / (1 + \beta N_{cf})$ . (A) Time course showing variation in both final steady-state force and rate 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.

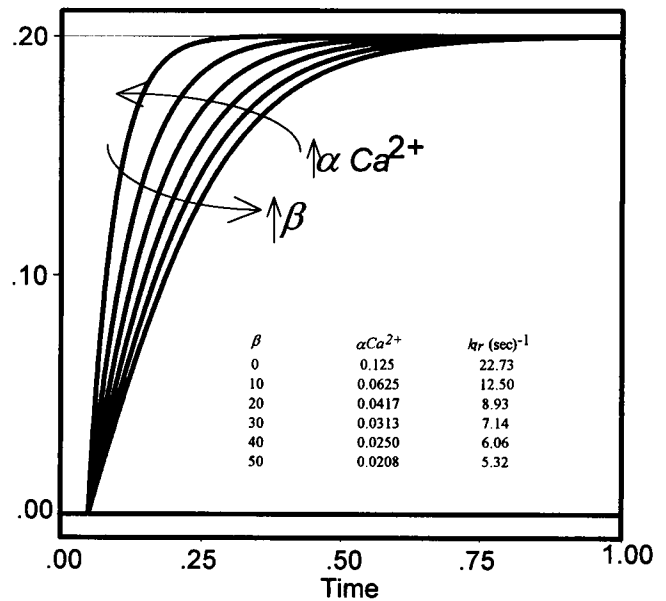


FIGURE 4 Varying degrees of cooperativity ( $\beta = 0, 10, 20, 30, 40, 50$ ) and corresponding varying levels of  $Ca^{2+}$  activation to produce in each case the same steady-state force ( $F_{ss} = 0.2F_{max}$ ).  $k_{tr}$  and the time course of force development decrease as cooperativity increases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

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  $6.06 \text{ s}^{-1}$  ( $\ll g$ ) at the lowest level of activation to  $27.8 \text{ s}^{-1}$  (between  $g$  and  $f + g$ ) at the highest level (Fig. 3 A).

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 ( $k_{tr,an} = 32.0 \text{ s}^{-1}$  in Fig. 2 versus  $k_{tr} = 27.8 \text{ s}^{-1}$  in Fig. 3), whereas the rates of force development at the lowest level of activation are very different ( $k_{tr,an} = 21.8 \text{ s}^{-1}$  in Fig. 2 versus  $k_{tr} = 6.0 \text{ s}^{-1}$  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  $\beta$  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  $\alpha\text{Ca}^{2+}$  and  $N_{cf}$  are low enough that  $K/(K + 1) \ll 1$ . If  $\alpha\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  activation. The net result of these steady-state effects is that cooperativity causes a pronounced shift to the left of the force-log  $\alpha\text{Ca}^{2+}$  curve, as shown in Fig. 5 (obtained by solution of Eq. 8 for various values of  $\alpha\text{Ca}^{2+}$ ). (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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  than at high  $\text{Ca}^{2+}$ . 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  in Figs. 3 and 4.

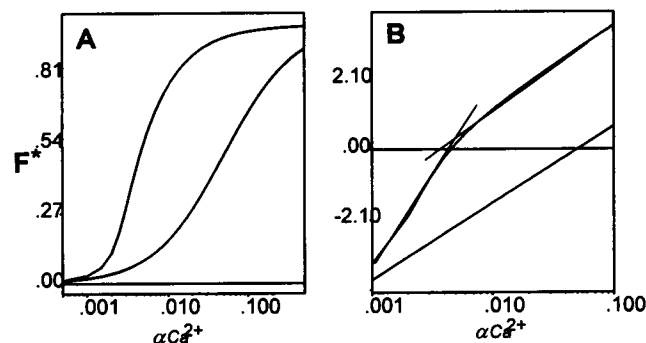


FIGURE 5 (A) Force-log  $\text{Ca}^{2+}$  curves for cases with (left curve) and without (right curve) cooperative feedback. (B) Same data as in A, but plotted with  $\log[F^*/(1 - F^*)]$  on the ordinate. Value 0.0 on the ordinate represents  $F^* = 0.5$ . Steeper slope for negative than for positive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

## DISCUSSION

### Cooperativity slows force development in a $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  activation is low and becomes less obvious when  $\text{Ca}^{2+}$  activation is high. The net impact is to make the rate constant of force development  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  in contrast to the fact that the rate constant characterizing the tension transient after phosphate release was not sensitive to  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ , 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  $\text{Ca}^{2+}$  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  $\beta$ . 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 evi-

dence (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 ( $< 5 \text{ s}^{-1}$ ) 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  $\text{Ca}^{2+}$  activation; they are most pronounced at low levels of  $\text{Ca}^{2+}$  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 $\text{Ca}^{2+}$ -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  $k'$ ,  $k'_{\text{on}}$ , and the off constant,  $k_{\text{off}}$ . Myosin heads can attach (strongly bind) to the thin filament only when the regulatory unit is in the "on" position. 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  $N_6$ ).  $\text{Ca}^{2+}$  binds and dissociates from the low-affinity TnC site on the regulatory unit with rate constants of association,  $k_+$ , and dissociation,  $k_-$ . When  $\text{Ca}^{2+}$  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.,  $k' \ll k_{\text{off}}$ ). When  $\text{Ca}^{2+}$  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.,  $k_{\text{on}} > k_{\text{off}}$ ).  $\text{Ca}^{2+}$  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 probability 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  $\text{Ca}^{2+}$  bound to TnC. In the absence of available  $\text{Ca}^{2+}$ ,  $N_6$  persists until cross-bridges 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  $k_{\text{off}} \gg f$ , and the greatest probability is that  $N_4$  will progress to  $N_1$ .

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

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

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

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

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

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

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

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,  $N_{\text{nc}}$ ; combine the two cycling but non-force-bearing states ( $N_3$  and  $N_4$ ) into a single state,  $N_{\text{c0}}$ ; combine the two force-bearing states ( $N_5$  and  $N_6$ ) into a single force-bearing state,  $N_{\text{cf}}$ . This makes it possible to rewrite Eqs. A.1–A.6 as three equations:

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

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

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

where

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

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

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

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

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

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

Equation A.12 gives an explicit dependence of  $K$  on 1)  $\text{Ca}^{2+}$ ; 2) the "on"–"off" switching constants; and 3) the  $\text{Ca}^{2+}$  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.

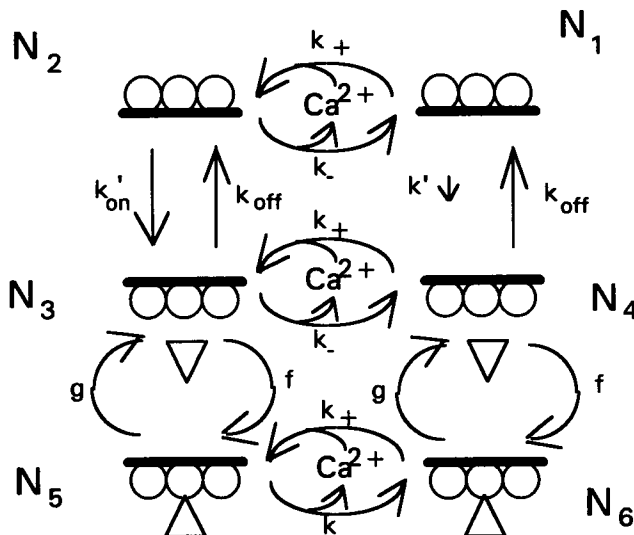


FIGURE 6 Six-state cross-bridge activation and cycling model. See text for definitions and explanations.

## 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  $\text{Ca}^{2+}$  binding constant ( $k_+$ ) relative to the  $\text{Ca}^{2+}$  dissociation constant ( $k_-$ ), i.e., they decrease  $\text{Ca}_{1/2}$ . 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  $\alpha_1$  is the zero  $N_{cf}$  switching constant and  $\beta_1$  is a cooperativity coefficient. Similarly, cooperativity by which force-bearing cross-bridges enhance  $\text{Ca}^{2+}$  binding may be represented by

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

where  $\alpha_2$  is the zero  $N_{cf}$   $\text{Ca}^{2+}$ -binding constant and  $\beta_2$  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:

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

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

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

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

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

Both  $K_0$  and  $K_1$  depend on  $\alpha \text{Ca}^{2+}$ , the cooperative factor  $\beta$ , and the value chosen for  $N_0$ . However,  $K_1$  has a stronger dependence on  $\beta$  than does  $K_0$ , such that strong cooperativity (i.e., high  $\beta$ ) tends to increase  $K_1$  more than  $K_0$ . Furthermore, at low  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ .

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

$$\dot{N}_{cf} = -[g + f(K_0 - AK_1)]N_{cf} - fK_1 N_{cf}^2 + fK_0 N_T. \quad (\text{A.16})$$

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  $\eta$ , gives

$$\dot{\eta} = -[g + f(K_0(1 + 2N_0) - K_1 N_T)]\eta + \delta, \quad (\text{A.17})$$

where  $\delta$  is some incremental part of  $fK_0 A$ . In this linearized equation, an incremental  $k_{tr}$  may be defined as

$$k_{trinc} = g + f(K_0(1 + 2N_0) - K_1 N_T). \quad (\text{A.18})$$

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

$$g < k_{trinc} < g + f$$

if

$$0 < K_0(1 + 2N_0) - K_1 N_T < 1 \quad (\text{A.19})$$

$$k_{trinc} < g$$

if

$$K_1 N_T > K_0(1 + 2N_0).$$

Both ranges are possible. At low  $\text{Ca}^{2+}$  activation (low  $N_0$ ) and strong cooperativity (high  $\beta$ ), it can be shown that  $K_1 N_T$  is relatively greater than  $K_0(1 + 2N_0)$ , with the result that  $k_{trinc}$  is even less than  $g$ . Such a low value of  $k_{trinc}$  means that force develops more slowly than one would expect from even the slowest step in the cross-bridge cycle. At high levels of  $\text{Ca}^{2+}$  activation,  $K_0(1 + 2N_0)$  begins to approach  $K_1 N_T$  and  $k_{trinc}$  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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## REFERENCES

- Araujo, A., and J. W. Walker. 1994. Kinetics of tension development in skinned cardiac myocytes measured by photorelease of  $\text{Ca}^{2+}$ . *Am. J. Physiol.* 267:H1643-H1653.
- Bremel, R. D., and A. Weber. 1972. Cooperation within actin filaments in vertebrate skeletal muscle. *Nature*. 238:97-101.
- Brenner, B. 1988. Effect of  $\text{Ca}^{2+}$  on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: implication for regulation of muscle contraction. *Proc. Natl. Acad. Sci. USA*. 85:3265-3269.
- Dobrunz, L. E., P. H. Backx, and D. T. Yue. 1995. Steady-state  $[\text{Ca}^{2+}]_i$ -Force relationship in intact twitching cardiac muscle: direct evidence for modulation by isoproterenol and EMD 53998. *Biophys. J.* 69:189-201.
- Gao, W. D., Y. Liu, R. Mellgren, and E. Maraban. 1996. Intrinsic myofilament alterations underlying the decreased contractility of stunned myocardium. A consequence of  $\text{Ca}^{2+}$ -dependent proteolysis? *Circ. Res.* 78:455-465.
- 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.
- Hancock, W. O., D. A. Martyn, and L. L. Huntsman. 1993.  $\text{Ca}^{2+}$  and segment length dependence of isometric force kinetics in intact ferret cardiac muscle. *Circ. Res.* 73:603-611.



- Hill, T. L. 1983. Two elementary models for the regulation of skeletal muscle contraction by calcium. *Biophys. J.* 44:383–396.
- Hoffman, P. A., and F. Fuchs. 1987. Evidence for a force-dependent component of calcium binding to cardiac troponin C. *Am. J. Physiol.* 253(*Cell Physiol.* 22):C541–C546.
- Landesberg, A., and S. Sideman. 1994. Coupling calcium binding to troponin C and cross-bridge cycling in skinned cardiac cells. *Am. J. Physiol.* 266(*Heart Circ. Physiol.* 35):H1260–H1271.
- Lehrer, S. S. 1994. The regulatory switch of the muscle thin filament:  $\text{Ca}^{2+}$  or myosin heads? *J. Muscle Res. Cell Motil.* 15:232–236.
- McDonald, K. S., L. J. Field, M. S. Parmacek, M. Soonpaa, J. M. Leiden, and R. L. Moss. 1995. Length dependence of  $\text{Ca}^{2+}$  sensitivity of tension in mouse cardiac myocytes expressing skeletal troponin C. *J. Physiol. (Lond.)* 483:131–139.
- Metzger, J. M., and R. L. Moss. 1990. pH modulation of the kinetics of a  $\text{Ca}^{2+}$ -sensitive crossbridge state transition in mammalian single skeletal muscle fibers. *J. Physiol. (Lond.)* 428:751–761.
- Millar, N. C., and E. Homsher. 1990. The effect of phosphate, and calcium on force generation in glycerinated rabbit skeletal muscle fibers. *J. Biol. Chem.* 265:20234–20240.
- Morimoto, S., and I. Ohtsuki. 1994. Role of troponin C in determining  $\text{Ca}^{2+}$ -sensitivity and cooperativity of the tension development in rabbit skeletal and cardiac muscles. *J. Biochem.* 115:144–146.
- Moss, R. L. 1992.  $\text{Ca}^{2+}$  regulation of mechanical properties of striated muscle. Mechanistic studies using extraction and replacement of regulatory proteins. *Circ. Res.* 70:865–884.
- Peterson, J. N., W. C. Hunter, and M. R. Berman. 1991. Estimated time course of  $\text{Ca}^{2+}$  bound to troponin C during relaxation in isolated cardiac muscle. *Am. J. Physiol.* 260(*Heart Circ. Physiol.* 29):H1013–H1024.
- Solaro, R. J., and J. Van Eyk. 1996. Altered interactions among thin filament proteins modulate cardiac function. *J. Mol. Cell. Cardiol.* 28:217–230.
- Swartz, D. R., and R. L. Moss. 1992. Influence of strong-binding myosin analogue on calcium-sensitive mechanical properties of skinned skeletal muscle fibers. *J. Biol. Chem.* 267:20497–20506.
- Sweitzer, N. K., and R. L. Moss. 1990. The effect of altered temperature on  $\text{Ca}^{2+}$  sensitive force in permeabilized myocardium, and skeletal muscle. *J. Gen. Physiol.* 96:1221–1245.
- Wang, Y. P., and F. Fuchs. 1994. Length, force, and  $\text{Ca}^{2+}$ -troponin C affinity in cardiac, and slow skeletal muscle. *Am. J. Physiol.* 266(*Cell Physiol.* 35):C1077–C1082.
- Wolf, M. R., K. S. McDonald, and R. L. Moss. 1995. Rate of tension development in cardiac muscle varies with level of activator calcium. *Circ. Res.* 76:154–160.
- Yue, D. T. 1987. Intracellular  $[\text{Ca}^{2+}]$  related to rate of force development in twitch contraction of heart. *Am. J. Physiol.* 252(*Heart Circ. Physiol.* 21):H760–H770.