A MODEL OF FORCE PRODUCTION THAT EXPLAINS THE LAG BETWEEN CROSSBRIDGE ATTACHMENT AND FORCE AFTER ELECTRICAL STIMULATION OF STRIATED MUSCLE FIBERS

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ABSTRACT Whereas the mechanical behavior of fully activated fibers can be explained by assuming that attached force-producing crossbridges exist in at least two configurations, one exerting more force than the other (Huxley A. F., and R. M. Simmons. 1971. *Nature* [*Lond.*]. 233:533–538), and the behavior of relaxed fibers can be explained by assuming a single population of weakly binding rapid-equilibrium crossbridges (Schoenberg, M. 1988. *Biophys. J.* 54:135–148), it has not been possible to explain the transition between rest and activation in these terms. The difficulty in explaining why, after electrical stimulation of resting intact frog skeletal muscle fibers at 1–5°C, force development lags stiffness development by more than 15 ms has led a number of investigators to postulate additional crossbridge states. However, postulation of an additional crossbridge state will not explain the following three observations: (*a*) Although the lag between force and stiffness is very different after stimulation, during the redevelopment of force after an extended period of high velocity shortening, and during relaxation of a tetanus, nonetheless, the plots of force versus stiffness in each of these cases are approximately the same. (*b*) When the lag between stiffness and force during the rising phase of a twitch is changed nearly fourfold by changing temperature, again the plot of force versus stiffness remains essentially unchanged. (*c*) When a muscle fiber is subjected to a small quick length change, the rate constant for the isometric force recovery is faster when the length change is applied during the rising phase of a tenanus than when it is applied on the plateau. We have been able to explain all the above findings using a model for force production that is similar to the 1971 model of Huxley and Simmons, but which makes the additional assumption that the force-producing transition envisioned by them is a cooperative one, with the back rate constant of the force-producing transition decreasing as more crossbridges attach.

INTRODUCTION

Since the pioneering work of A. F. Huxley and H. E. Huxley, which showed that muscle contraction is due to crossbridges that project from the myosin filament and interact with actin (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954; Huxley, 1957), much work has been devoted to determining the states of the crossbridge during the contraction cycle and relating this to the mechanism of force generation. From studies of the force transient seen after an abrupt change of length of an activated muscle, Huxley and Simmons (1971) suggested that the force-generating step in contracting muscle involved a rapid transition of the attached crossbridge between at least two conformationally different configurations. The first configuration was postulated to form with a rate constant on the order of f in the Huxley, 1957 scheme (~15 s^{-1}); the last was thought to detach with a rate constant on the order of g in the 1957 scheme (~100 s^{-1}). The two attached crossbridge configurations were thought to interconvert with rate constants in the neighborhood of 10^3 s^{-1}. Although definitive proof of this scheme has been lacking, it nonetheless forms the basis of much of the current thinking concerning the steps involved in force generation.

From studies of relaxed muscle (Brenner et al., 1982; Schoenberg et al., 1984), a second type of attached crossbridge, the so-called "weakly binding rapid-equilibrium" crossbridge, was postulated. Crossbridges in this state were found to bind to actin more weakly than the crossbridges postulated by Huxley and Simmons, and were found to attach to and detach from the actin filament with rate constants in the neighborhood of 10^3–10^4 s^{-1} (Schoenberg, 1988*a*,*b*). Just as crossbridges behaving as described by Huxley and Simmons (1971) were adequate for explaining much of the behavior of activated fibers, weakly binding rapid-equilibrium crossbridges explained most of the behavior of relaxed fibers.

While the resting and activated condition have individually been well explained, the transition between rest and activation has not been so well explained. Stiffness and x-ray diffraction measurements suggest that after electrical stimulation of frog skeletal muscle at 0°C, crossbridge attachment precedes force generation by ~15 ms (Bressler and Clinch, 1974; Cecchi et al., 1982; Tamura et al., 1982; Schoenberg and Wells, 1984; Ford et al., 1986; Kress et al., 1986; Bagni et al., 1988; Bressler et al., 1988). Whereas the model of Huxley and Simmons (1971) predicts a lag between stiffness and force, the predicted lag is only on the order of a millisecond. This is very different from the 10–15-ms lag seen experimentally.

This discrepancy between the model of Huxley and Simmons and experiment has led a number of investigators (Huxley and Kress, 1985; Ford et al., 1986) to postulate the existence of extra crossbridge states beyond those postulated by Huxley and Simmons (1971) and Brenner et al. (1982). Whereas postulating an additional state can explain the lag between stiffness and force on the rising phase of a tetanus, it does not readily explain several other observations. It does not explain why the lag between stiffness and force seen during the rising phase of a tetanus is different from the lag between stiffness and force seen during the redevelopment of force after an extended period of high velocity shortening, and also different from the lag between force and stiffness seen during the fall (relaxation) of the isometric tetanus. It does not explain why, even though the lag between stiffness and force in each of the above instances is different, plots of force versus stiffness for each of the above cases are exceedingly similar (Cecchi et al., 1987; Bagni et al., 1988). Nor does it explain why, when the temporal relationship between stiffness and force during the rising phase of a twitch is changed by varying temperature, again the plot of stiffness versus force remains approximately constant (Schoenberg, M., and J. B. Wells, unpublished observations). Finally, a fourth observation not explained by existing models is that when one abruptly decreases the length of an activated muscle fiber during the rising phase of a tetanus or during the plateau of a tetanus, the rate of the isometric force recovery in the first instance is significantly faster than in the second (Bagni et al., 1985, 1987; Ford et al., 1986).

The failure of existing models, even with postulation of additional states, to simply explain the above findings has led us to search for an alternative explanation for the available data. We have found we can explain all the above findings using a model that is similar to the 1971 model of Huxley and Simmons, but which has the additional assumption that the force-producing transition envisioned by them is a cooperative one. In addition to offering explanation for a large number of seemingly diverse observations, this model has the virtue of (*a*) being quite testable, and (*b*) not requiring postulation of crossbridge states beyond those previously postulated by Huxley and Simmons (1971) and Brenner et al. (1982).

METHODS

Computational

In their 1917 model, Huxley and Simmons postulated that force generation was a result of crossbridges attaching to actin in a lightly strained, low-force configuration and then undergoing a rapid, force-producing conformational change to a more highly strained configuration. Mathematically, this is represented as

$$B \underset{k_{cb}}{\overset{k_{bc}}{\rightleftharpoons}} C,$$

where B stands for crossbridges in the low-strain configuration C, for crossbridges in the high-strain configuration, and k_{bc} and k_{cb} are the forward and reverse rate constants for the force-producing conformational change. Here we are interested in the transition between rest and activation and the scheme must include the relaxed state so we have

$$A \underset{k_{ba}}{\overset{k_{ab}}{\rightleftharpoons}} B \underset{k_{cb}}{\overset{k_{bc}}{\rightleftharpoons}} C,$$

where, in addition to the symbols defined above, A stands for crossbridges in the relaxed state, and k_{ab} and k_{ba} are the forward and back rate constants for the transition between the relaxed state and the force-producing state of Huxley and Simmons (1971).[1]

When we calculated the behavior of the Huxley-Simmons model, the rate constants of transition between configurations B and C were taken directly from Huxley and Simmons (1971). Thus k_{bc} was $200 \cdot \exp(-y/2)$ and k_{cb} was $200\ s^{-1} \cdot y$ is the change in crossbridge extension relative to y_o, y_o being the extension of the crossbridge in the isometric state when the crossbridge is midway through the B to C transition. As in Huxley and Simmons (1971), the calculations are performed only for crossbridges having the single extension, $y_o = 8$ nm. In the cooperative model developed, k_{bc} was the same as in Huxley and Simmons (1971), but k_{cb} was modified as described in the Results section. Details of k_{ab} and k_{ba} also are given in the Results section.

Although the above equations are possibly simple enough to solve analytically, this was not attempted and instead the equations were solved numerically using Gear's method (Gear, 1971). The necessary computations were performed on a Masscomp 5500, a 68010-based supermicrocomputer, and each calculation typically required 30–60 s of computation.

Experimental

For Figs. 1, 2, and 4, single intact fibers from frog lumbricalis digiti IV muscle were prepared as described by Bagni et al. (1988). They were tetanized once very 4 min with pulses that were 0.5 ms in duration and 1.5 times threshold in strength. Length changes were measured at the sarcomere level either by means of a laser diffraction system (Bagni et al., 1988) or a striation follower (Huxley et al., 1981). In some experiments, sarcomere length was kept constant during contraction using the servo system described by Bagni et al. (1988). Stiffness was measured by oscillating the fibers ~0.1% at 4–6 kHz and calculating the ratio of force

[1] In this paper we use the word state in its broadest sense. Thus we consider the crossbridges to be in one of two states: either in the weakly binding, rapid-equilibrium crossbridge state that predominates in the unstimulated fiber (Schoenberg, 1988, *a* and *b*), or the stiff, force-producing crossbridge state that predominates in the active fiber (Huxley and Simmons, 1971). The force-producing crossbridges, from Huxley and Simmons (1971), exist in two configurations, herein referred to as B and C. Relaxed crossbridges also have two configurations, attached and detached, but for the work here, the attached configuration may be ignored.

to sarcomere displacement. In all figures, only data where the phase difference between force and displacement was negligible are included.

For the experiments of Fig. 3, small bundles of frog semitendinosus fibers were stimulated with a single 0.5-ms duration pulse whose amplitude was 1.5 times threshold. Stiffness and force were measured as in Schoenberg and Wells (1984).

RESULTS

The experimental results whose lack of explanation formed the impetus for development of the model presented here are illustrated in Figs. 1–4. The most important and well known of these, which is shown in Fig. 1 *A*, is that after electrical stimulation of frog skeletal muscle at 1–5°C, stiffness, and presumably crossbridge attachment, precedes force development by 15–20 ms (Bressler and Clinch, 1974; Cecchi et al., 1982, 1987; Tamura et al., 1982; Schoenberg and Wells, 1984; Ford et al., 1986; Kress et al., 1986; Bagni et al., 1988; Bressler et al., 1988). A second finding also not well understood at present concerns the different relationships between stiffness and force during the rise of a tetanus, during the redevelopment of

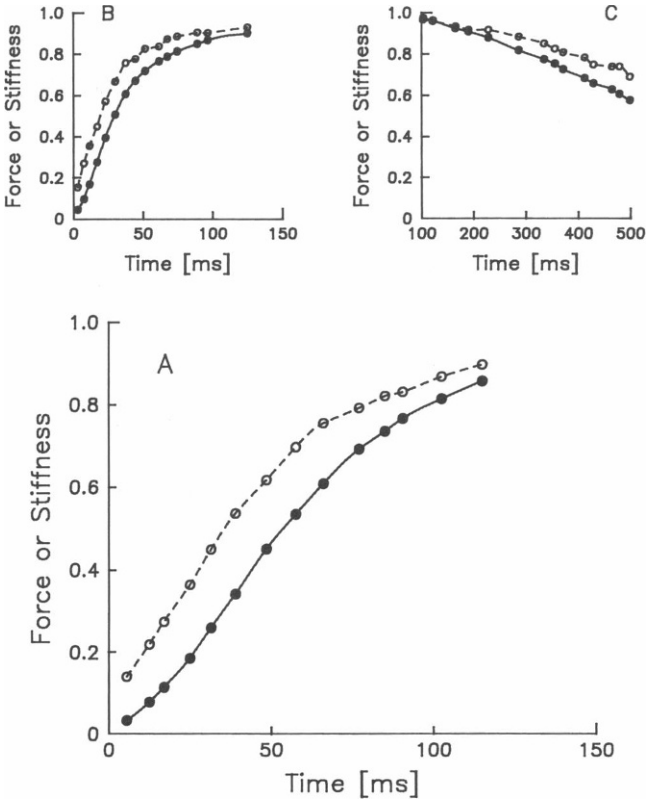

FIGURE 1 The temporal relationship between force (*solid curve*) and stiffness (*dashed curve*) in intact frog lumbricales fibers at 4°C. (*A*) Rising phase of the tetanus, stimulation starting at $t = 0$. (*B*) Redevelopment of force after a 5% rapid decrease in length of a fully activated fiber. (*C*) Relaxation (falling) phase of a tetanus (stimulation ceasing at $t = 0$). Stiffness was measured with a 4 kHz sinusoidal length oscillation of 0.1% amplitude. In *B*, the 5% quick decrease in length meant that before the period of force redevelopment displayed, the fiber had shortened at near maximal velocity for ~25 ms.

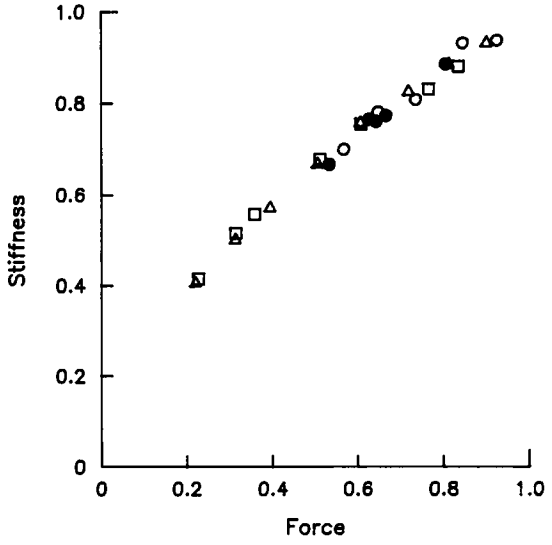

FIGURE 2 Force versus stiffness during the rising phase of a tetanus, during the redevelopment of force after an extended period of high velocity shortening, and during relaxation of a tetanus. Force and stiffness values are normalized to the force and stiffness on the plateau of a tetanus. *Squares*, rising phase; *triangles*, redevelopment of force after an extended period of high velocity shortening as in Fig. 1 *B*; *circles*, relaxation phase, two experiments. The actual time lag between stiffness and force at a relative force of 0.5 was 17.5 ms for the rising phase, and 10 ms for the rerise after shortening. For relaxation, the lag between force and stiffness at a relative force of 0.6 was ~85 ms. Data from frog lumbricales fibers, 4°C. Stiffness measured with a 4-kHz length oscillation of ~0.1% amplitude.

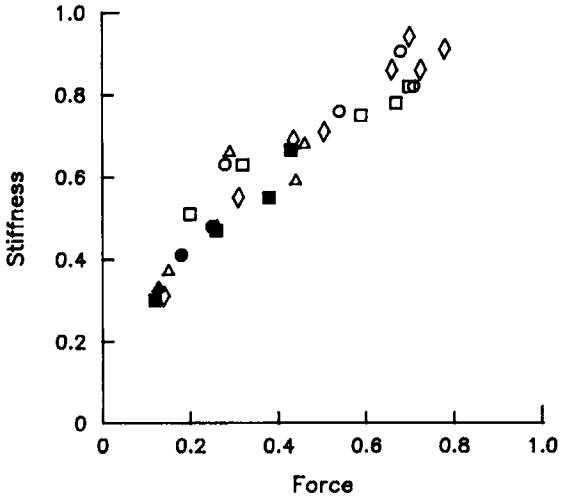

FIGURE 3 Force versus stiffness during the rising phase of twitches generated at different temperatures. Force and stiffness values are normalized to the force and stiffness on the plateau of the tetanus. *Open symbols*, twitches at 6°C; *solid symbols*, twitches at 26° or 31°C. Different symbols represent fiber bundles from different frog semitendinosus muscles. The two points obtained at 31°C (*diamonds*) are not readily visualized because, by coincidence, they practically overlay the two lowest solid square symbols. The stiffness–force relationships appear similar at all the temperatures studied even though the temporal lag between stiffness and force is 19.3 ms at 26°C and as little as 5.1 ms at 6°C. Stiffness was measured from the propagation speed of a mechanical distrubance as described in Schoenberg and Wells (1984).

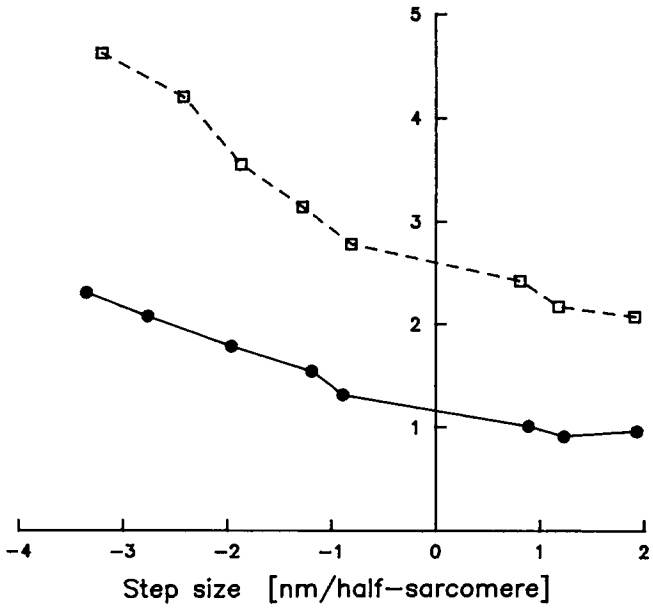

FIGURE 4 The rate constant, in reciprocal millisecond, of the fast force recovery seen after a small quick release or stretch of an activated fiber on the plateau and rising phase of a tetanus. Important features are: the larger the release size, the faster the rate constant for force recovery; and for any given size stretch or release, the rate constant on the rising phase is faster than on the plateau. *Squares*, rising phase, $T_0/P_{max} \approx 0.5$, where P_{max} = maximum isometric tension. *Circles*, plateau. Frog tibialis anterior, 6°C. Step time, 120 μs. Negative step sizes connote releases, positive ones connote stretches. The rate constants were calculated as $0.693/t_{1/2}$, where $t_{1/2}$ is the halftime for the rapid force recovery.

force after an extended period of high velocity shortening, and during the relaxation of a tetanus. While the magnitude of the lag in milliseconds in each of these cases is different (Fig. 1), plots of force versus stiffness are very much the same (Fig. 2, see also Cecchi et al., 1986, 1987; Bagni et al., 1988). Similar results are also seen when one stimulates twitches at different temperatures. Changing the temporal lag between stiffness and force during a twitch nearly fourfold by increasing the temperature from 6° to 26°C (Schoenberg and Wells, 1984), again has, as shown in Fig. 3, little effect on the force–stiffness relationship. A final finding in need of explanation, illustrated in Fig. 4, is the finding that the rate constant for the rapid force recovery after a small quick release of an activated fiber is faster during the rising phase of the tetanus than it is on the plateau (Bagni et al., 1985, 1988; Ford et al., 1986).

As stated in the Introduction, to explain the above data we have developed a model that is a modification of that proposed by Huxley and Simmons (1971). The first step, therefore, is to reproduce their results. For simplicity, we use only the simplest model they considered, the model where force production is the result of a transition between just two conformationally different attached crossbridge configurations. We refer to these two configurations as simply configuration B and configuration C.

To model the rise of a tetanus, we make the assumption that for $t < 0$ (resting condition), all the crossbridges are in state A, i.e., the rate constant for the transition from A to B, k_{ab}, is zero.[2] For $t > 0$, under the assumption that at $t = 0$ Ca^{2+} is released, it is assumed that k_{ab} increases to 15 s^{-1}. For, simplicity, the reverse rate constant, k_{ba}, is fixed at 0.00001 s^{-1}. This means that on the tetanus plateau all the crossbridges will be in the attached states B and C. This approximation is made in keeping with the observation that these states predominate in the active fiber. The rate constants governing the transition between B and C, k_{bc} and k_{cb}, are, in the initial calculations, the same as those given in Huxley and Simmons, 1971 (see Methods). In the sections that follow, we first illustrate the behavior of the above Huxley-Simmons type model and then the behavior of an alternative model.

The Huxley-Simmons Model

Fig. 5 shows the rise of force, and also stiffness, for the Huxley-Simmons type model described above. In Fig. 5, as well as the remaining figures, force and stiffness are normalized to their peak values. Comparison of Fig. 5 with Fig. 1, or Fig. 6 of Ford et al. (1986) shows that taking $k_{ab} = 15$ s^{-1} for $t > 0$, gives a value for the halftime of stiffness development similar in magnitude to that seen experimentally. However, it is also seen that the Huxley-Simmons type model produces only a small lag, on the order of 1 ms, between stiffness and force on the rising phase, not the 15–20-ms lag seen experimentally. This result is a basic property of the Huxley-Simmons model, it is not a consequence of the particular way in which we have modeled activation. Allowing k_{ab} to go from 0 to 15 s^{-1} over a 50-ms interval, rather than instantaneously, again results in the same small lag between stiffness and force (calculations not shown).

To further examine the behavior of the Huxley-Simmons type model, we calculated its response to different size small quick releases or stretches. These were imposed both on the plateau of the force response, at t = 300 ms, and also at the middle of the rising phase (t = 50 ms). Typical results are shown in Fig. 6. In agreement with experiment, and as expected from a Huxley-Simmons type model, the response to a quick decrease in fiber length is an instantaneous force drop followed by a rapid force recovery. Also in agreement with the model of Huxley and Simmons and experiment, the larger the size of the quick release, the faster the rate constant of the rapid force recovery. Using the nomenclature of Huxley and Simmons, we define T_0 as the force before the length change, T_1 as the force immediately after the length change, and T_2 as the approximate value of the force seen after the quick

[2]Although, for complete correctness, state A should be divided into both an attached and detached component, for simplicity, the attached component can safely be ignored since, for the conditions studied here, the attached component would not contribute very much to either force or stiffness (see Schoenberg, 1988, *a* and *b*).

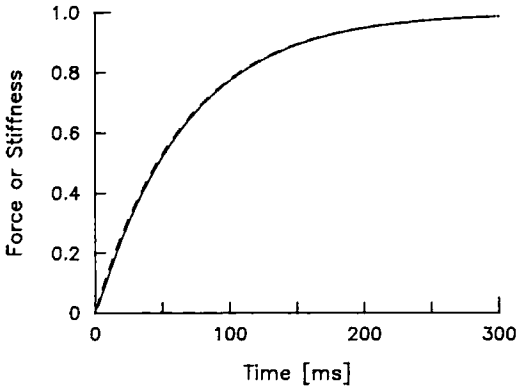

FIGURE 5 Rise of force (*solid curve*) and stiffness (*dashed curve*) for a Huxley-Simmons type model. Activation and other parameters are described in text. Stiffness precedes force at the half-maximal point by 1.3 ms. A more realistic, i.e., more sigmoidal shape for the rise of force and stiffness is obtained by allowing k_{ab} to go from 0 to 15 s^{-1} over a 50-ms interval, rather than instantaneously. However, here too the lag between force and stiffness is also about the same (calculations not shown).

phase of the tension recovery. To estimate T_2 during the rising phase, where the force is changing even in the absence of an imposed quick length change, the response that would occur in the absence of a length change was subtracted from the response with the length change. It is clear that we have successfully programmed the Huxley-Simmons equations since our values of T_1/T_0 and T_2/T_0 at the tetanus plateau, and also our values for the rate constant of the tension recovery on the tetanus plateau agree precisely with those given in Figs. 4 and 8 of Huxley and Simmons (1971). The values of the rate constants are shown in Fig. 7. This figure not only shows the agreement with Fig. 4 of Huxley and Simmons (1971), it also illustrates another important point. For a Huxley-Simmons type model, the rate constant of the rapid force recovery is the same during the rise of the tetanus as it is on the plateau. This does not agree with the experimental result seen in Fig. 4 here and also reported in Bagni et al. (1985; 1988) and Ford et al. (1986).

It is clear then, that while the 1971 model of Huxley and Simmons describes quite well the mechanical behavior of a fully activated fiber, it does not explain very well the transition between rest and activation. Specifically, it does not explain the faster rate constant for the rapid force recovery seen during the rising phase of a tetanus nor does it explain the rather large lead of stiffness over force seen during the tetanus rise.

The fact that stiffness precedes force during the rising phase of a tetanus means essentially that the force per attached crossbridge, that is, the force per unit stiffness, is less during the rising phase than it is during the plateau. It is therefore instructional to consider what determines the force per crossbridge during the plateau and rising phase. In the model of Huxley and Simmons, crossbridges attached in configurations B and C both have the same stiffness. Yet crossbridges in configuration B exert less force than those in configuration C. Therefore, one thing that influences the force per crossbridge in models similar to the Huxley-Simmons model is the ratio of the number of bridges in configuration C to the number in configuration B. This ratio, in turn, depends upon the free energy difference between configurations B and C. Thus, one way

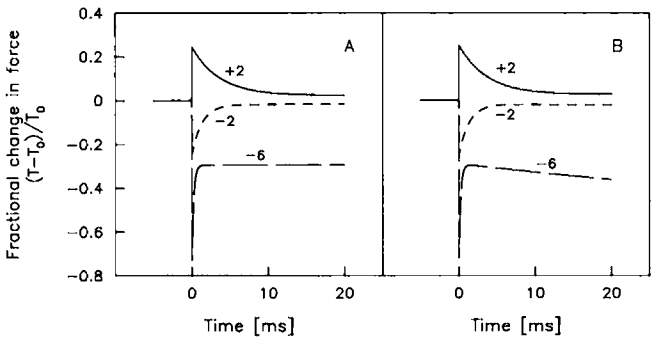

FIGURE 6 Typical responses to a rapid stretch or release for the Huxley-Simmons type model. T stands for force, T_0 for the force at the time of the release or stretch. Numbers next to each curve give the size of release (−) or stretch (+) in nm/half-sarcomere. (*A*) Responses at t = 300 ms, plateau of tetanus. (*B*) Responses at t = 50 ms, rising phase, where T_0/P_{max} = 0.5 (P_{max} = maximum isometric tension). In *B*, the tension response in the absence of a length change was subtracted from the response with the length change in order to extract the response due solely to the stretch or release which would otherwise be superimposed on the background rise in force. Note the basic similarity in the amplitude and rate constants of the responses on the plateau and on the rising phase.

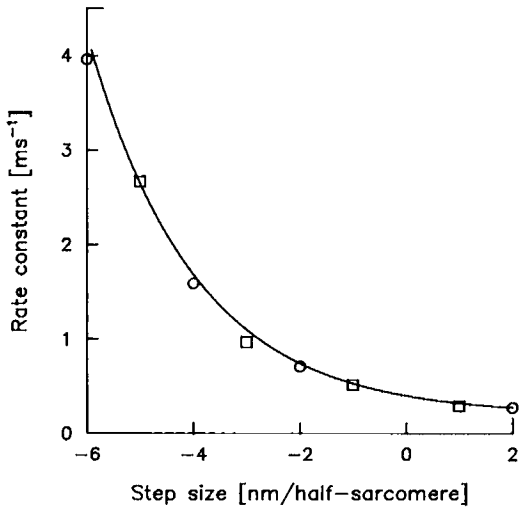

FIGURE 7 Rate constants for the rapid force recovery of the Huxley-Simmons type model as determined from records like those in Fig. 6. Solid line is from Huxley and Simmons (1971). *Circles*, rate constants for release or stretch during plateau (t = 300 ms). *Squares*, rate constants for releases or stretches at t = 50 ms (when T_0/P_{max} is 0.52). The rate constants were calculated as $0.693/t_{1/2}$, where $t_{1/2}$ is the halftime for the rapid force recovery. Note that the rate constants for the rapid force recovery on the rising phase of the simulated tetanus are virtually identical to those on the plateau.

of making the force per crossbridge be greater on the plateau of a tetanus than on the rising phase, would be to have the free energy difference between B and C increase as more crossbridges attach. We will make use of this in developing the model below.

A Model of Force Production that Will Explain the Lag between Stiffness and Force on the Rising Phase

In this section we show that a small but significant modification of the Huxley-Simmons model for force generation allows one to explain virtually all of the experimental findings explained by the Huxley-Simmons model and also those illustrated in Figs. 1–4. As hinted at above, the modification we make is to have the difference in minimum free energy between the configurationally different states B and C increase as crossbridges attach. To say that the free energy difference between B and C increases as more crossbridges attach is the same as saying that the step governing the transition between the configurations B and C shows positive cooperativity. This is because having the free energy difference between states B and C increase as more crossbridges attach means that the equilibrium between B and C shifts towards C as more crossbridges attach. The increase in the free energy between B and C could be accompanied either by a relative increase in k_{bc} or a relative decrease in k_{cb}. It will become clear that either of these alternatives will explain the data of Figs. 1–3 but to explain the data of Fig. 4 it is necessary that k_{cb} decrease.

To emphasize that the difference between the model we will develop here and the model of Huxley and Simmons is basically whether the force-producing transition is cooperative or not, in our new model we keep all the parameters identical to the Huxley-Simmons parameters except k_{cb}. Instead of keeping k_{cb} fixed at 200 s^{-1} as in the Huxley-Simmons model, we set it equal either to 1,600 − 1,400 · $(n_B + n_C)$ s^{-1}, where n_B and n_C are the fraction of crossbridges in configurations B and C respectively, or, in a second example, to 800 − 600 · $(n_B + n_C)$ s^{-1}. Thus we examine the effect of having k_{cb} decrease either four- or eightfold as the number of attached crossbridges increases from 0 to 100%.

The results of these calculations are shown in Figs. 8–10. As seen from Fig. 8, our cooperative model gives a rate of stiffness development identical to that of the Huxley-Simmons type model of Fig. 5. However, unlike the model of Fig. 5, the new model now correctly has force lagging stiffness by 15–30 ms. One very significant aspect of the new model is that, on any time scale greater than a few milliseconds, the relationship between stiffness and force depends not upon time or level of activation, but upon the number of attached crossbridges. As a result, if k_{ab} for $t >$ 0 were 30 s^{-1} rather than 15 s^{-1}, this would, as expected, reduce the halftime for stiffness development from 46 to 23 ms and it would reduce the lag between stiffness and force for the case where k_{cb} changes fourfold from 18 to 9 ms. However, importantly, the relationship between stiffness and force would remain constant. The relationship between stiffness and force for both the 4× and 8× change in k_{cb} is shown in Fig. 9. As a point of comparison, the experimental data of Fig. 2 are also shown.

One would also expect to see the same behavior described above during the relaxation phase of a tetanus. When we modeled relaxation by making k_{ab} zero and allowing crossbridges to detach from state C with a rate constant of 5 s^{-1} (calculations not shown), this gave a

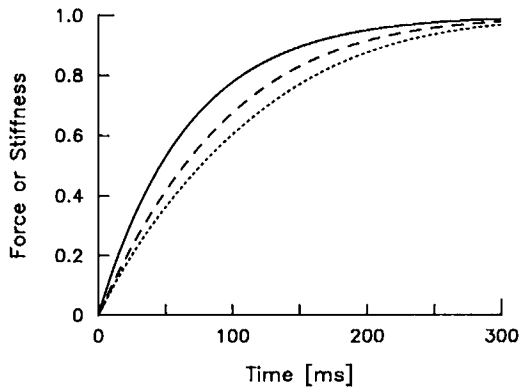

FIGURE 8 The rise of force and stiffness for the model in which the force-generating step is cooperative. All parameters are the same as in the model of Fig. 5 with the exception of k_{cb}. *Dashed curve*, rise of force when k_{cb} = 800 − 600$(n_B$-$n_C)$ s^{-1}. *Dotted curve*, rise of force when k_{cb} = 1,600 − 1,400$(n_B$-$n_C)$ s^{-1}. *Solid curve*, the stiffness rise in both cases, is also identical to the stiffness rise for the Huxley-Simmons model of Fig. 5.

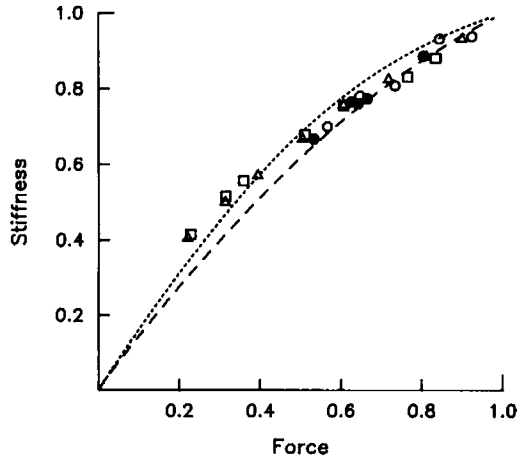

FIGURE 9 The relationship between force and stiffness for the model of Fig. 8. Dashed and dotted curves are for the fourfold and eightfold change in k_{cb}, respectively, as connoted in Fig. 8. For both cases, the force–stiffness relationship obtained was independent of whether it was determined from the rise of a tetanus (modeled by k_{ab} = 15 s^{-1}), the rise of force after an extended period of high velocity shortening (modeled by k_{ab} = 30 s^{-1}), or during the relaxation of force (modeled by k_{ab} = 0, k_{cb} = 5 s^{-1}). Also superimposed upon the theoretical curves are the data from Fig. 2.

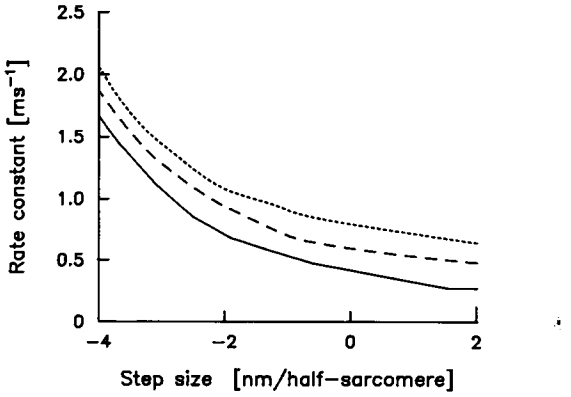

FIGURE 10 Rate constants of the rapid force recovery on the plateau and rising phase of the simulated tetanus for the model of Fig. 8. *Solid curve*, calculated rate constants on the plateau of the tetanus (t = 400 ms), for either a four- or eightfold change in k_{cb}. *Dashed curve*, rate constants for the rapid force recovery for the case where k_{cb} changes fourfold. *Dotted curve*, similar curve for the model where k_{cb} changes eightfold. For dashed curve, the sudden length changes were at 64 ms; for dotted curve, the sudden length changes were at 76.5 ms. These times were chosen so that at the time of the quick release or stretch, the force was half the maximal tetanic value. For both these models, the rate constants of the rapid force recovery on the plateau of the simulated tetanus (*solid curve*) were the same as those calculated for the Huxley-Simmons model of Fig. 5.

halftime for force relaxation of 255 ms and a halftime for stiffness decay of 390 ms. Yet, the relationship between stiffness and force was still the same as that shown in Fig. 9. This key feature of our model corresponds very much to the experimental situation, as seen from Figs. 2 and 3.

A final point with regard to our model is that it also offers an explanation for the finding that the rate constant for force recovery after a quick release is higher during the the rising phase of the tetanus than on the plateau. The rate constant for force recovery in our cooperative model, as well as the Huxley-Simmons model, is on the order of $(k_{bc} + k_{cb})$. The reason we chose to make the B to C transition cooperative by having k_{cb} rather than k_{bc} vary is because this results in k_{cb} being larger during the rising phase than during the plateau. This then causes the rate constants for force recovery with the cooperative model to be identical to those of the Huxley-Simmons model on the plateau, but to be larger on the rising phase. The calculated results are shown in Fig. 10 and comparison to Fig. 4 shows that the model qualitatively predicts the experimentally observed changes.

DISCUSSION

It has been known for some time that after electrical stimulation of frog skeletal muscle at rest, crossbridge attachment, as measured by stiffness or equatorial x-ray ratio, increases more rapidly than force (Bressler and Clinch, 1974; Cecchi et al., 1982, 1986; Tamura et al., 1982; Schoenberg and Wells, 1984; Ford et al., 1986; Kress et al., 1986; Bressler et al., 1988; Bagni et al., 1988). Because of tendon and other compliance, in most of the early studies a certain amount of sarcomere shortening occurred after activation. This could have contributed to the delay in force relative to crossbridge attachment, since if force decreases more with sarcomere shortening than does attached crossbridge number, as in the Huxley 1957 scheme, then sarcomere shortening per se should cause a delay in force relative to stiffness. In recent studies, however, the sarcomere shortening subsequent to stimulation has been largely eliminated, and these studies still show a significant lag, on the order of 15 ms at 0°C, between stiffness and force. This lag then appears to be a fundamental property of the crossbridge interaction with actin.

In the 1971 Huxley-Simmons model of force generation, after electrical stimulation, crossbridges attach to actin in a lightly strained, low-force configuration and then undergo a rapid conformational change to a highly strained, high-force configuration. Since the crossbridges attach first in the low-force configuration and since both configurations have the same stiffness, this scheme has stiffness developing more rapidly than force during the rise of a tetanus. However, the lag between stiffness and force is only on the order of a millisecond, this being approximately the reciprocal of the sum of forward and reverse rate constants for the transition between the two configurations. For this reason, it is clear that the Huxley-Simmons scheme as formulated in 1971 will not explain the 15-ms lag between stiffness and force during the rising phase of a tetanus.

One potential possibility for explaining this 15-ms lag came from the discovery of the ability of weakly binding crossbridges in a relaxed fiber to attach to the actin filament (Brenner et al., 1982; Schoenberg, 1988, *a* and *b*). These so-called M · ATP crossbridges could potentially explain the lag between stiffness and force because they contribute much more to fiber stiffness than to force. However, at present the best evidence suggests that in intact relaxed frog fibers, these crossbridges spend <5% of their time attached to actin (Schoenberg, 1988, *a* and *b*). Thus these crossbridges would be expected to contribute only a little to the lead of stiffness over force after electrical stimulation.

The fact that the 15-ms lead of stiffness over force after stimulation is explained neither by the 1971 Huxley-Simmons model nor by the attachment of M · ATP crossbridges had led a number of investigators to postulate additional types of crossbridge states. Huxley and Kress (1985) postulated that there was a type of relaxed crossbridge with properties different from that observed by Brenner et al. (1982). Whereas the M · ATP crossbridges observed by Brenner et al. attached to actin at physiological ionic strength with a rate constant greater than 10^2 s^{-1} (Schoenberg, 1988, *a* and *b*), Huxley and Kress envisioned a type of relaxed crossbridge that would attach to actin with a rate constant of only 15 s^{-1}. They suggested that

these crossbridges, which attached initially in a configuration that produced little force, would then convert to a high-force configuration with a rate constant of $\sim 100\ s^{-1}$.

Ford et al. (1986) also attempted to explain the lag between force and stiffness. In contrast to Huxley and Kress, they felt that the relaxed crossbridges were probably of the type described by Brenner et al. (1982). However, they too suggested an additional state, one which was postulated to occur in the crossbridge cycle after the M · ATP state of Brenner et al. and before the force-producing state of Huxley and Simmons (1971). Again this new state was postulated (*a*) to produce little force and (*b*) to convert to a force-producing state with a rate constant of $\sim 100\ s^{-1}$.

Both the scheme of Huxley and Kress and that of Ford et al. will explain the lag between stiffness and force after electrical stimulation. However, both schemes postulate additional states for which there is little independent physiological or biochemical evidence. In addition, neither of these schemes explains the finding of Cecchi et al. (1987) that while the lag between stiffness and force is different during the rising phase of a tetanus, during redevelopment of force after an extended period of high velocity shortening, and during the isometric relaxation of a tetanus (Fig. 1), nonetheless, plots of force versus stiffness in each of these instances are virtually identical (Fig. 2). Nor do these schemes explain the observation (Fig. 3) that during twitch contractions at different temperatures, the lag between stiffness and force changes but, again, the stiffness–force plot does not. In the present calculations we have shown that one can, in fact, explain all the above findings without postulating new states. What is needed is to postulate that the transition between the lightly strained and highly strained configurations envisioned by Huxley and Simmons is a cooperative one. Our results suggest that as little as a four- to eightfold change in the equilibrium constant between these configurations as the number of crossbridges goes from 0 to 100% will explain the relationship between force and attachment during the experimental situations enumerated above.

The amount of cooperativity between attached configurations necessary to explain the various lags is not excessively large. At physiological ionic strength, S1·ADP, the S1 moiety often thought to be an analogue of the force-producing state in fibers, binds to regulated actin with a binding constant of $\sim 5 \times 10^5\ M^{-1}$ (Greene, 1982). Assuming that the actin concentration in solution necessary to mimic the fiber situation is ~5 mM (Brenner et al., 1986; Pate and Cooke, 1988), the free energy of binding of the ADP crossbridge should be ~8 kT. The approximately fivefold change in k_{cb} necessary to explain the various lags between stiffness and force corresponds to a free energy difference of 1.6 kT, a value only 20% of 8 kT.

It should be pointed out that not only does the cooperative model developed here explain all the various lags between force and stiffness, but if, compatible with the idea of a cooperative force-producing transition, the back rate constant of that transition decreases as more crossbridges attach, then the model described will also offer an explanation for the finding that the response to a quick length change imposed during the rise of a tetanus has a faster time-course then the response to a length change imposed on the plateau. This is a consequence of the fact that the rate constant for the fast redevelopment of force after a length change is on the order of $(k_{bc}+k_{cb})$. In the model of Huxley and Simmons this sum of rate constants is fixed, but in the model developed here, k_{cb} decreases as the number of attached crossbridges increases. It appears then, that making the force-producing transition envisioned by Huxley and Simmons cooperative gives a model that retains the strong points of the Huxley-Simmons model while at the same time making up for some of its deficiencies.

We are not the first to suggest that the producing transition might be cooperative. It has been known for some time that when myosin subfragment 1 binds to naked or regulated actin in solution, the binding occurs as a two-step process (Trybus and Taylor, 1980, 1982; Coates et al., 1985). The first moiety formed upon attachment is a weakly binding one, which then isomerizes to a strongly binding moiety. Geeves et al. (1984) and Geeves and Halsall (1987) postulated that this isomerization step might be cooperative in the presence of troponin and tropomyosin and also that it might correspond to the force-generating step during contraction. Although the specific model of Geeves et al. (1984) and Geeves and Halsall (1987) need not be correct in order for the model we have presented here to be valid, that model is clearly compatible with the one presented here, and vice versa. In that regard, we point out that the model of Geeves et al. (1984) will readily explain the data of Figs. 1–3, and, if the cooperativity they describe occurs through a change in the back rate of the weak- to strong-binding isomerization, that model would then essentially be identical to the one presented here and also explain the data of Fig. 4.

It behooves us to ask what independent evidence exists for a cooperative force transition. It has long been known that the actual binding of crossbridges in fibers and also myosin in solution to actin having troponin and tropomyosin can be cooperative (Bremel and Weber, 1972; Reuben et al., 1971; Greene and Eisenberg, 1980). Eisenberg and Hill (1985) have suggested that crossbridges fall into two broad categories; weakly binding ones that show almost no cooperativity in their binding to regulated actin (such as the S1·ATP crossbridge), and strongly binding ones that do show cooperativity in binding (such as rigor S1). While lumping of the crossbridges into two broad categories appears valid as a first approximation, the recent biochemical evidence supports the idea of a continuum in the amount of cooperativity in binding of different species. This is based upon the observation that the amount of cooperativity in binding among species seems to follow the

order S1·ATP < pPDM-treated S1 < S1·AMP-PNP < S1·ADP < rigor S1 (Greene et al., 1986; Greene and Eisenberg, 1980; Greene, 1982). One interesting observation about this continuum is that, for some unknown reason, the degree of cooperativity seems to correlate with the strength of binding of the species. If one can conclude from this that troponin–tropomyosin should have a greater influence on the strength of binding of the more strongly binding C configuration that upon the less strongly binding B configuration, then one might speculate that it is not unreasonable that the free energy difference between B and C increase as additional crossbridges attach. The argument is as follows: When many crossbridges are attached to the actin filament and troponin–tropomyosin is the turned-on form (Güth and Potter, 1987; Gordan et al., 1988; Williams et al., 1988), troponin–tropomyosin should have little effect upon the binding of either configuration B or C. However, when fewer crossbridges are attached and troponin–tropomyosin goes into the turned-off form (Güth and Potter, 1987; Gordon et al., 1988; Williams et al., 1988), this presumably might cause a greater weakening in the binding of C than in the binding of B. This is based on the unexplained correlation outlined above. Since C in the first place binds more tightly than B, this greater weakening in the binding of C will result in the free energy difference between configurations B and C being less when fewer crossbridges are attached.

In summary, we have proposed a model of force production that retains all the advantages of the Huxley-Simmons model, and yet, at perhaps some sacrifice of simplicity, explains a much wider range of phenomena. Our model has the desirable feature that it enables one to explain the properties of relaxed and activated fibers, as well as the transition between rest and activation, without postulating any new states beyond those postulated by Huxley and Simmons (1971) and Brenner et al. (1982). The key feature of our model is that the force-producing transition is cooperative; details beyond that should not be taken too literally. Thus, in our modeling we have chosen to make the back rate constant vary with the total number of strongly attached crossbridges, but rather similar behavior would be predicted if the back rate constant were to vary with the number of attached crossbridges in configuration C, with the number in configuration B, or even with the total force. Furthermore, while we have chosen to keep our model as close as possible to that of Huxley and Simmons, even this is not essential. If force generation was due, not to a conformational change in S1, but to the subfragment 2 domain of the myosin molecule lifting up from the myosin filament backbone and melting (Harrington, 1979), our model would then essentially be suggesting that the greater the number of S2 domains lifted up from the filament backbone, the greater the desire of S2 to melt and generate force. Only additional experimental evidence can distinguish between these various interesting possibilities.

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