Roles of Ca\textsuperscript{2+} and Crossbridge Kinetics in Determining the Maximum Rates of Ca\textsuperscript{2+} Activation and Relaxation in Rat and Guinea Pig Skinned Trabeculae

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Abstract—We examined the influences of Ca\textsuperscript{2+} and crossbridge kinetics on the maximum rate of force development during Ca\textsuperscript{2+} activation of cardiac myofibrils and on the maximum rate of relaxation. Flash photolysis of diazo-2 or nitrophenyl-EGTA was used to produce a sudden decrease or increase, respectively, in [Ca\textsuperscript{2+}] within Triton-skinned trabeculae from rat and guinea pig hearts (22°C). Trabeculae from both species had similar Ca\textsuperscript{2+} sensitivities, suggesting that the rate of dissociation of Ca\textsuperscript{2+} from troponin C (k\textsubscript{off}) is similar in the 2 species. However, the rate of relaxation after diazo-2 photolysis was 5 times faster in the rat (16.1±0.9 s\textsuperscript{-1}, mean±SEM, n=11) than in the guinea pig (2.99±0.26 s\textsuperscript{-1}, n=7). This indicates that the maximum relaxation rate is limited by crossbridge kinetics rather than by k\textsubscript{off}. The maximum rates of rapid activation by Ca\textsuperscript{2+} after nitrophenyl-EGTA photolysis (k\textsubscript{on}) and of force redevelopment after forcible crossbridge dissociation (k\textsubscript{on}) were similar and were ≈5-fold faster in rat (k\textsubscript{on}=14.4±0.9 s\textsuperscript{-1}, k\textsubscript{on}=13.0±0.6 s\textsuperscript{-1}) than in guinea pig (k\textsubscript{on}=2.57±0.14 s\textsuperscript{-1}, k\textsubscript{on}=2.69±0.30 s\textsuperscript{-1}) trabeculae. This too may be mainly due to species differences in crossbridge kinetics. Both k\textsubscript{on} and k\textsubscript{on} increased as [Ca\textsuperscript{2+}] increased. This Ca\textsuperscript{2+} dependence of the rates of force development is consistent with current models for the Ca\textsuperscript{2+} activation of the crossbridge cycle, but these models do not explain the similarity in the maximal rates of activation and relaxation within a given species. (Circ Res. 1998;83:179-186.)

Key Words: crossbridge ■ photolysis ■ Ca\textsuperscript{2+} activation ■ cardiac myofibril ■ relaxation

Cardiac muscle contraction is initiated by Ca\textsuperscript{2+} binding to troponin C (TnC), which triggers structural changes in the thin filament and allows actomyosin crossbridges to generate force. However, relatively little is known about the precise mechanism by which Ca\textsuperscript{2+} regulates crossbridge force generation. In the original “steric blocking” model,\textsuperscript{1} Ca\textsuperscript{2+} was considered to act as a switch controlling the number, but not the kinetics, of cycling crossbridges. However, Brenner\textsuperscript{2} showed that Ca\textsuperscript{2+} accelerates crossbridge kinetics, as measured by the rate of force redevelopment (k\textsubscript{on}) after forcible detachment of the crossbridges during Ca\textsuperscript{2+} activation. He therefore proposed a kinetic model\textsuperscript{1} in which Ca\textsuperscript{2+} activates both force and k\textsubscript{on} by increasing the rate of transition of crossbridges from the non–force-generating (detached or weakly bound) state to the force-generating state (f\textsubscript{det}). On the other hand, it has been suggested more recently\textsuperscript{3-5} that a Ca\textsuperscript{2+} dependence of k\textsubscript{on} is also consistent with the steric blocking model if the rate of crossbridge attachment is influenced by the activation of the thin filament by strongly binding crossbridges. Thus, the fact that k\textsubscript{on} is found to be Ca\textsuperscript{2+} dependent in skeletal fibers\textsuperscript{3,4,6} does not distinguish between these models. In cardiac muscle, however, Hancock and colleagues\textsuperscript{7,8} found that k\textsubscript{on} was independent of [Ca\textsuperscript{2+}], which is consistent only with the steric blocking model in the Ca\textsuperscript{2+} regulation of cardiac muscle. At present, this is the source of some controversy, since other groups have found k\textsubscript{on} to be activated by Ca\textsuperscript{2+} in cardiac muscle.\textsuperscript{9-11} In the present study, we addressed this controversy by examining the Ca\textsuperscript{2+} dependence of k\textsubscript{on} in skinned cardiac muscles using an optimized length change protocol.

Little is also known about the factors that govern the kinetics of Ca\textsuperscript{2+} activation and of relaxation of cardiac myofibrils, although these are likely to be important in determining the normal duration of the heartbeat. Theoretically, the maximum rates of activation and relaxation could be governed by (1) the rate at which Ca\textsuperscript{2+} binds to or dissociates from TnC or (2) the rate of crossbridge cycling. A major aim of the present study was therefore to examine the relative roles of Ca\textsuperscript{2+} and crossbridges in determining the intrinsic rates of myofibrillar activation and relaxation rates, by comparing these rates in rat myofibrils with those in guinea pig myofibrils, which have much slower crossbridge kinetics\textsuperscript{12,13} but similar myofibrillar Ca\textsuperscript{2+} sensitivities\textsuperscript{13} (and probably, therefore, similar kinetics of Ca\textsuperscript{2+} interaction with TnC). The measurement of the intrinsic rates of myofibrillar activation or relaxation has recently been made possible by development of “caged” Ca\textsuperscript{2+}. © 1998 American Heart Association, Inc.
compounds, which can be photolyzed to either release or chelate Ca\(^{2+}\) after they have diffused into the myofibrils. Photolysis of these compounds causes solution [Ca\(^{2+}\)] to change in \(\approx\)1 millisecond, so the subsequent force change takes place at a rate that is limited by the intrinsic properties of the myofibrils rather than by the rate of change of [Ca\(^{2+}\)]. To study the relaxation rate, we used the “caged” chelator of Ca\(^{2+}\), diazo-2,13,14 whereas for the activation rate, we used the “caged” Ca\(^{2+}\) compound, nitrophenyl-EGTA (NP-EGTA).16,17

A comparison of the maximum rate of Ca\(^{2+}\) activation of the thin filament (\(k_{\text{act}}\)) with the maximum \(k_{\text{rel}}\) can provide information on whether Ca\(^{2+}\)-activated force development is limited by crossbridge kinetics rather than by \(k_{\text{rel}}\). This was previously achieved using the caged Ca\(^{2+}\), nitr-7,11 but this compound did not lead to full activation of the myofibrils. We therefore repeated these experiments using NP-EGTA, which releases sufficient Ca\(^{2+}\) so that the maximal activation is achieved and \(k_{\text{rel}}\) can be compared with \(k_{\text{act}}\). Our experiments also allowed the first comparison of maximum \(k_{\text{rel}}\) and relaxation rates in the same muscles. The above crossbridge models predict that the relaxation rate should be slower than the activation rate, and our experiments allowed us to test this.

**Materials and Methods**

**Preparations and Equipment**

Animals were supplied by Bantin & Kingman Universal Ltd (Hull, UK). The general procedure and apparatus were as described in detail previously.15,16 In summary, male Wistar rats (\(\approx\)250 g) were stunned by a blow to the head and then killed by cervical dislocation. Male Dunkin Hartley guinea pigs (\(\approx\)200 g) were dissected from the right ventricles in Tyrode’s solution containing 25 mmol/L K\(_2\) EGTA, 10 mmol/L Ca\(^{2+}\), and 0.1 mmol/L phenylmethylsulfonyl fluoride. A trabecula was then mounted in monofilament snares attached to an isometric force transducer (AE801, SensoNor) and to a servomotor (300S, Cambridge Technology Inc). The muscle bath system included a bath of reduced volume (10 \(\mu\)L) specially designed for photolysis experiments.15 Bath temperature was 22°C or 12°C (\(\pm\)0.1°C). Sarcomere length (SL) was measured by laser diffraction and was set to \(\approx\)2.1 \(\mu\)m in the relaxed muscle at 22°C.

Diazo-2 and NP-EGTA were photolyzed by light from a xenon flashlamp (Hi-Tech Ltd), filtered with a UG5 bandpass filter (300 to 400 nm). The light was focused, and its area of incidence on the muscle was optimized as described previously.16 PClamp software (Axon Instruments) was used to trigger the flashlamp or to impose rapid length changes on the trabeculae for \(k_{\text{rel}}\) measurements (see below). Force, muscle length, and SL were recorded on a 4-channel chart recorder and on a computer using a 12-bit analog/digital board sampling at 2 kHz.

**Solutions**

All solutions contained (mmol/L) BES 100, Na\(_2\)H\(_2\)ATP 6.3, Na\(_2\) phosphate 10, MgCl\(_2\) 5.4 to 6.2 (to maintain [Mg\(^{2+}\)] at 1 mmol/L), potassium phosphate 1, glutathione 5, leupeptin 0.001, and potassium propionate 50 to 80 (to give an ionic strength of 200 mmol/L) (pH 7.1). Details of solution manufac-
confirming the results of Ventura-Clapier et al.13 In addition, the relaxation of guinea pig trabeculae followed a double-exponential curve, the initial fast phase of which represented the maximum intrinsic rate of myofibrillar relaxation.14,15 Trabeculae were activated in solutions containing Ca\(^{2+}\) and 0.25 mmol/L diazo-2 before the flash (160 mJ) was triggered. Force was normalized between the mean preflash value and the mean force between 990 and 1000 milliseconds. In this example, preflash and postflash forces in the rat were 0.40 and 0.10 (relative to maximum force) and in the guinea pig were 0.61 and 0.33, respectively. Relaxations were fitted with single-exponential (for guinea pig) or double-exponential (for rat) curves (solid lines).

**Rapid Relaxation of Myofibrils**

We used flash photolysis of diazo-2, a caged chelator of Ca\(^{2+}\), to produce a sudden drop in solution [Ca\(^{2+}\)] and thereby measure the intrinsic rate of myofibrillar relaxation.14,15 Trabeculae were activated in solutions containing Ca\(^{2+}\) and diazo-2, and once force was steady the diazo-2 was photolyzed (Figure 2). Relaxation of rat trabeculae followed a double-exponential curve, the initial fast phase of which represents the maximum intrinsic rate of myofibrillar relaxation. However, the guinea pig relaxation was fitted best by a single-exponential curve. As found with rat muscles,15 the relaxation rate of guinea pig trabeculae was independent of both preflash [Ca\(^{2+}\)] and force (results not shown).

As shown in Figure 2, guinea pig trabeculae relaxed much more slowly than did rat trabeculae. The rate constants for the 2 phases of relaxation in rat muscles were 16.1±0.9 s\(^{-1}\) and 0.97±0.13 s\(^{-1}\) (n=11) at 22°C and that for guinea pig muscles was 2.99±0.26 s\(^{-1}\) (n=7). The maximum rate of myofibrillar relaxation in rat myofibrils was therefore 5 times faster (P<0.01) than that of the guinea pig. This difference was not due to the different fitting protocols, because fitting the rat data with a single-exponential fit (over the first 200 milliseconds) gave a rate constant of 18.9±0.9 s\(^{-1}\) (n=11, results not shown).

It could be argued that both muscles had a slow phase of relaxation but that only the rat muscle also had a fast phase; however, this is untenable because the rate constant for the slow phase in the rat was significantly less (P<0.01) than the guinea pig single rate constant. The origin of the slow phase of the biexponential relaxation remains unclear.

**Rapid Activation of Myofibrils**

The rate constant for Ca\(^{2+}\) activation of force, k\(_{act}\), in the trabeculae was measured using flash photolysis of the “caged” Ca\(^{2+}\) compound, NP-EGTA, to produce a sudden elevation of solution [Ca\(^{2+}\)].16,17 As shown in Figure 3A, photolysis of NP-EGTA produced rapid activation of force in trabeculae from both species, and full Ca\(^{2+}\) activation from the resting state was achieved with a 160-mJ flash. In some cases, peak force (expressed relative to the maximum steady-state force) exceeded 1.0 in the few seconds after photolysis. This overshoot of force occurred in all the guinea pig muscles and in a subset of the rat muscles (Figure 3B and 3C). The reasons for the secondary decrease of force are unclear but could include time-dependent SL rearrangement or accumulation of Pi inside the muscles. The postflash rise in force was well fitted by a single-exponential curve, with a k\(_{act}\) at maximum flash energy (160 mJ) of 14.4±0.9 s\(^{-1}\) (n=20) in the rat compared with 2.57±0.14 s\(^{-1}\) (n=8, P<0.01) in the guinea pig (Figure 3A). Thus rat trabeculae activated 5-fold faster than did trabeculae from guinea pigs. These rates were not

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**Figure 1.** Ca\(^{2+}\) sensitivities of skinned trabeculae from rat (A) and guinea pig (B). Forces are expressed relative to maximum force developed (at pCa 4.5). Sigmoidal curves were generated from the Hill equation using the mean values of pCa\(_{50}\) and n\(_{H}\). Points represent mean±SEM of 39 experiments for rat and 8 experiments for guinea pig.

**Figure 2.** Typical traces showing relaxation of rat and guinea pig skinned trabeculae after photolysis of diazo-2. Muscles were activated in Ca\(^{2+}\) and 0.25 mmol/L diazo-2 before the flash (160 mJ) was triggered. Force was normalized between the mean preflash value and the mean force between 990 and 1000 milliseconds. In this example, preflash and postflash forces in the rat were 0.40 and 0.10 (relative to maximum force) and in the guinea pig were 0.61 and 0.33, respectively. Relaxations were fitted with single-exponential (for guinea pig) or double-exponential (for rat) curves (solid lines).
to vary the degree of NP-EGTA photolysis. The peak force achieved after photolysis gave an indirect measure of the energy of the flash was altered between 50 and 160 mJ. Force was normalized between the mean preflash and peak postflash forces in the rat were 0.07 and 1.0 and in guinea pig were 0.02 and 1.0, respectively. Solid lines are single exponential fits ending at 98% of peak force. In panels B (rat) and C (guinea pig), the rate constant of the exponential rise in force ($k_{\text{act}}$) is plotted against the peak force achieved after photolysis. Peak force was varied by altering flash energy and is expressed relative to maximum steady-state force. Each symbol type refers to a different muscle.

Figure 3. Panel A shows typical traces of maximal activations of rat and guinea pig trabeculae produced by NP-EGTA photolysis (160 mJ). Force was normalized between the mean preflash value and the mean force between 1490 and 1500 milliseconds. Relative to maximum steady-state force, preflash and peak postflash forces in the rat were 0.07 and 1.0 and in guinea pig were 0.02 and 1.0, respectively. Solid lines are single exponential fits ending at 98% of peak force. In panels B (rat) and C (guinea pig), the rate constant of the exponential rise in force ($k_{\text{act}}$) is plotted against the peak force achieved after photolysis. Peak force was varied by altering flash energy and is expressed relative to maximum steady-state force. Each symbol type refers to a different muscle.

significantly different from the corresponding maximum rates of relaxation.

To determine whether $k_{\text{act}}$ varied with postflash [Ca$^{2+}$], the energy of the flash was altered between 50 and 160 mJ to vary the degree of NP-EGTA photolysis. The peak force achieved after photolysis gave an indirect measure of postflash [Ca$^{2+}$]. (Calculation of the postflash [Ca$^{2+}$] was precluded because the precise degree of photolysis of NP-EGTA was unknown.) Plotting this peak force against $k_{\text{act}}$ (Figure 3B and 3C) clearly demonstrated that the rate of activation increased with postphotolysis force (and hence [Ca$^{2+}$]) in trabeculae from both rat and guinea pig. In both cases, the relationship between $k_{\text{act}}$ and force was curved upward.

Rate of Redevelopment of Force

We investigated whether the difference in $k_{\text{act}}$ between species (Figure 3) was paralleled by a difference in crossbridge kinetics, as measured at constant [Ca$^{2+}$] by $k_{\text{tr}}$ after forcible detachment of the crossbridges. Crossbridges were detached by releasing the muscles by 20% (to allow unloaded shortening to occur) and then restretching them rapidly. SL was not controlled in these experiments, partly to make the results comparable to those from the flash photolysis experiments and partly because the sarcomere diffraction pattern was usually lost during maximal activation at 22°C. We found that the value of $k_{\text{tr}}$ depended on the duration of the holding period, becoming faster in both species as the duration of the holding period was shortened (Figure 4). This may reflect incomplete detachment of crossbridges at short holding durations. On the other hand, when the holding period was increased beyond 25 milliseconds for rat and 100 milliseconds for guinea pig, force started to redevelop during the holding period once the slack in the muscle had been taken up. Thus, we used different optimal holding periods (25 and 100 milliseconds for rat and guinea pig, respectively) to ensure that minimal numbers of crossbridges were attached before redevelopment of force after the restretch.

A comparison of $k_{\text{tr}}$ in rat and guinea pig is shown in Figure 5. Generally, the residual force (the point from which force redevelops after the length increase) was larger in the guinea pig than in the rat. Force redevelopment was well fitted by a single exponential curve in both species. The mean $k_{\text{tr}}$ during maximal activation (pCa 4.5) was 13.0±0.6 s$^{-1}$ for the rat (n=17) and 2.69±0.30 s$^{-1}$ for the guinea pig (n=9) (P<0.01). The large (4.5-fold) difference in $k_{\text{tr}}$ between species was not due to the different holding periods used, since $k_{\text{tr}}$ recorded from guinea pig trabeculae using a holding period of 25 milliseconds (as for rat) was 2.80±0.20 s$^{-1}$ (n=3), which was still much slower than $k_{\text{tr}}$ recorded from rat trabeculae (Figure 5). The $k_{\text{tr}}$ values above were not significantly different from the maximum values of $k_{\text{act}}$ measured in the same species. Thus, the different rates of Ca$^{2+}$ activation in the 2 species apparently reflect the relative rates of the force-generating processes.

The relationship between $k_{\text{tr}}$ and steady-state force was also determined for each species (Figure 5C and 5F) in order to compare the dependencies of $k_{\text{tr}}$ and of $k_{\text{act}}$ on force. Force was varied by altering the [Ca$^{2+}$] of the activating solutions. Because there was scatter in the data between muscles, particularly for the guinea pig (Figure 5F), to compare $k_{\text{tr}}$ values at different force levels we pooled data for each muscle. Values of $k_{\text{tr}}$ in the relative force range of 0.5 to 0.7 were not significantly different (P>0.05, paired t test) from those in the force range of 0.1 to 0.3 in either rat or guinea pig but were significantly lower (P<0.005) than maximal $k_{\text{tr}}$ values (relative force, 1.0) in both species. Thus, $k_{\text{tr}}$ was independent of force...
below ≈60% of maximum but increased at higher force levels. The data in Figure 5C and 5F were averaged and replotted as $k_\text{tr}$ against pCa (Figure 6). As [Ca\textsuperscript{2+}] rose from pCa 6.0 to 4.5, $k_\text{tr}$ increased 2-fold in the guinea pig and 10-fold in the rat. In both species, the pCa required to give 50% of the maximal $k_\text{tr}$ value (≈5.4, Figure 6) was less than the pCa 50 for force (5.6, Figure 1). Thus, force was more sensitive than $k_\text{tr}$ to Ca\textsuperscript{2+}, which may account for the downward curvature in the force-$k_\text{tr}$ relationships (Figure 5).

**Role of Compliance in Determining $k_\text{tr}$**

Brenner and Eisenberg\textsuperscript{19} showed that force redevelopment is slowed considerably if there is internal shortening in muscles due to end compliance. Thus, the slower force kinetics recorded in guinea pig compared with rat trabeculae could have been due to greater internal shortening in the guinea pig muscles. Although at 22°C the sarcomere pattern was usually lost during activation, at 12°C it was more stable, and we were able to measure SL during $k_\text{tr}$ determinations at 12°C in 1 guinea pig trabecula and 8 rat trabeculae. The resting SL was increased for these experiments (to 2.23±0.01 μm for rat [n=8 trabeculae] and 2.25 μm for the one guinea pig trabecula) to increase the SL during maximal activation, which helped to maintain the sarcomere pattern in the active muscle. As shown in Figure 7, force redevelopment at 12°C was much slower than at 22°C, but $k_\text{tr}$ was still markedly faster in the rat (6.41±0.51 s\textsuperscript{-1}, n=8) than in the guinea pig (0.97 s\textsuperscript{-1}, n=1). During force redevelopment, SL varied by <3% in both rat and guinea pig trabeculae. Thus, the 6-fold faster $k_\text{tr}$ value for the rat is unlikely to be explained by greater internal shortening in the guinea pig.

**Discussion**

Relaxation Rate Is Limited by Properties of Crossbridges

Theoretically, myofibrillar relaxation rate may be limited either by the rate at which Ca\textsuperscript{2+} dissociates from TnC ($k_{\text{off}}$) and causes thin filament deactivation (which may also be affected by the presence of strongly bound crossbridges; see below) or by the net rate at which myosin crossbridges detach from actin once Ca\textsuperscript{2+} is lost from TnC. The identical force-pCa relationships of the 2 species (Figure 1 and Reference 13) imply that $k_{\text{off}}$ should be similar in the 2 species. (Ca\textsuperscript{2+} sensitivity is largely determined by the Ca\textsuperscript{2+} affinity constant of TnC, which is equal to the ratio of the on and off rate constants for Ca\textsuperscript{2+} binding: affinity = $k_{\text{on}}/k_{\text{off}}$. Since $k_{\text{on}}$ has been reported to be diffusion-limited\textsuperscript{20} and therefore is constant, affinity may be determined chiefly by $k_{\text{off}}$.) Crossbridge kinetics, however, are known to be faster in the rat than in the guinea pig.\textsuperscript{12,13} Using photolysis of diazo-2
to measure the intrinsic relaxation rate of cardiac myofibrils, we found relaxation rates of 16.1 s$^{-1}$ and 2.99 s$^{-1}$, respectively, in rat and guinea pig trabeculae under identical conditions. This 5-fold difference in relaxation rate between the 2 species therefore suggests that the relaxation rate is limited not by $k_{off}$ but rather by the detachment rate of the crossbridges themselves.

**Figure 5.** Comparison of $k_{tr}$ in rat (A to C) and guinea pig (D to F) trabeculae. Muscles were given a step shortening of 25 or 100 milliseconds (A and D). Force was normalized to that before the length change, in this case maximum Ca$^{2+}$-activated force (B and E). Exponential curves were fitted to the first 1 second of the force redevelopment traces (superimposed solid lines). Panel E also illustrates that $k_{tr}$ increased only slightly when the holding period was decreased from 100 to 25 milliseconds in the guinea pig. In panels C and F, $k_{tr}$ is plotted against the force before the length change. The step was held for 25 milliseconds for rat and 100 milliseconds for guinea pig. Each symbol type refers to a different muscle.

**Activation Rate Is Determined by Properties of Crossbridges**

Although measurements were made without SL control, and internal shortening could have slowed force development, our $k_{tr}$ value of 13.0 s$^{-1}$ at 22°C in maximally activated rat trabeculae was similar to, or faster than, that found in other studies with SL control when differences in temperature were taken into account. $k_{act}$, which reflects the rate of crossbridge reattachment and transition to force-generating states, was measured under constant activation by Ca$^{2+}$, thus bypassing any direct influence of [Ca$^{2+}$] or species on the rate at which Ca$^{2+}$ triggers conformational changes in the regulatory proteins of the thin filament. Our finding that in a given species, and under identical conditions, $k_{act}$ is similar to $k_{tr}$ suggests that the maximum rate of Ca$^{2+}$-activated force development is limited by the rate at which detached or weakly bound crossbridges enter the force-generating state rather than by the rate at which the thin filament is switched on by Ca$^{2+}$ binding to TnC.

Overall, we found that the maximum values for $k_{act}$, $k_{tr}$, and the rate of relaxation were ≈5 times faster in rats compared with guinea pigs, even when internal shortening was minimal (Figure 7). It seems likely that the different activation and
relaxation rates of the 2 species relate to differences in their crossbridges. Rat ventricle contains mostly V1 myosin (composed of α-myosin heavy chains), whereas in guinea pig V2 and V3 myosins (αβ- and ββ-myosin heavy chains) predominate.12 Unpublished experiments (1997), performed by Dr P. Reiser (Ohio State University), showed that our rat muscles contained 80% to 90% α-myosin heavy chains (ie, mostly V1 myosin), whereas guinea pig contained only 40% to 50% α-myosin heavy chains. In general, crossbridges composed of V1 myosin have a 2- to 6-fold higher cycling rate than crossbridges with V3 myosin, as judged by various biochemical and mechanical assays.12,13,21,22 The faster crossbridge kinetics of V1 compared with V3 myosin probably explain a large part of the faster activation and relaxation rates in rats compared with guinea pigs, although there may be other species differences that lead to differing crossbridge kinetics between the corresponding myosin isoforms.

Another factor that could potentially explain, or at least contribute to, the species-specific difference in maximal activation and relaxation rates is the cooperative activation of the thin filament by the attachment of strongly binding crossbridges.3–5 This phenomenon tends to slow $k_{tr}$, particularly at low [Ca$^{2+}$].5 Thus, if cooperative activation due to attachment of strong-binding crossbridges were greater in guinea pig myofilaments than in rat myofilaments, this could explain why $k_a$, $k_{act}$, and possibly the rate of relaxation were slower in the guinea pig. However, we think this explanation is unlikely, because (1) this effect of strongly binding crossbridges is small at maximal Ca$^{2+}$ activation15 and thus would have little effect on the maximum values of $k_a$ and $k_{act}$, and (2) enhanced cooperativity of strongly bound crossbridges in guinea pig myofilaments would tend to make the force-pCa relationship steeper for guinea pig than for rat, yet there was no evidence for this (Figure 1).

**$k_{act}$ and $k_{tr}$ Are Ca$^{2+}$ Dependent**

The values of $k_{act}$ and $k_a$ increased as Ca$^{2+}$-activated force rose above 60% of maximum in both rat and guinea pig trabeculae (Figures 3, 5, and 6). There is currently controversy over a possible Ca$^{2+}$ dependence of $k_a$ in cardiac muscle. Hancock and colleagues7,8 reported no change in $k_{tr}$ when Ca$^{2+}$ was varied in intact7 and skinned8 cardiac muscles, whereas a Ca$^{2+}$ dependence of $k_a$ was observed by other groups using rat skinned trabeculae9 and myocytes.10,11 In the present study, we confirm that $k_{tr}$ is increased as [Ca$^{2+}$] rises. Possible reasons why Hancock and colleagues7,8 reached the opposite conclusion have been discussed elsewhere5,8–10; one difference is that some of their experiments used a length change with a minimal holding period, which leads to a higher value (Figure 4), and possibly a Ca$^{2+}$ independence, of $k_{tr}$. Our results (Figure 4) show the importance of defining the optimal holding period for $k_a$ measurements. Although we found that $k_a$ was activated by Ca$^{2+}$ in both species, this was less for the guinea pig (~2-fold, Figure 6) than for the rat (~10-fold), which is similar to the observation that activation of $k_a$ is greater in fast skeletal than in slow skeletal fibers.6 The significance of the Ca$^{2+}$ dependence of $k_a$ (and $k_{act}$) remains to be clarified: as with previous studies, our finding that $k_a$ and $k_{act}$ are Ca$^{2+}$ dependent could support...
In conclusion, (1) the relaxation rate is not limited by the rate of dissociation of Ca$^{2+}$ from TnC but reflects the kinetics of the crossbridges, (2) faster myofibrillar activation and relaxation rates in rat trabeculae compared with guinea pig trabeculae most likely relate to myosin type, and (3) the similarity of the rates of relaxation and activation (or force redevelopment) in a given species is difficult to reconcile with previous crossbridge models for the Ca$^{2+}$ regulation of $k_b$ but may be explained if a reversal of the crossbridge working stroke contributes to relaxation.

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### References


