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

Sue Palmer, Jonathan C. Kentish

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{act}) and of force redevelopment after forcible crossbridge dissociation (k\textsubscript{tr}) were similar and were 5-fold faster in rat (k\textsubscript{act}=14.4±0.9 s\textsuperscript{-1}, k\textsubscript{tr}=13.0±0.6 s\textsuperscript{-1}) than in guinea pig (k\textsubscript{act}=2.57±0.14 s\textsuperscript{-1}, k\textsubscript{tr}=2.69±0.30 s\textsuperscript{-1}) trabeculae. This too may be mainly due to species differences in crossbridge kinetics. Both k\textsubscript{act} and k\textsubscript{tr} 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{tr}) after forcible detachment of the crossbridges during Ca\textsuperscript{2+} activation. He therefore proposed a kinetic model\textsuperscript{3} in which Ca\textsuperscript{2+} activates both force and k\textsubscript{tr} 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{sep}). On the other hand, it has been suggested more recently\textsuperscript{4-5} that a Ca\textsuperscript{2+} dependence of k\textsubscript{tr} 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{tr} is found to be Ca\textsuperscript{2+} dependent in skeletal fibers\textsuperscript{2,6} does not distinguish between these models. In cardiac muscle, however, Hancock and colleagues\textsuperscript{7,8} found that k\textsubscript{tr} 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{tr} 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{tr} 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+}...
compartments, which can be photolysed 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,$^{14,15}$ 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 $k_{\text{act}}$. 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 $k_{\text{act}}$ can be compared with $k_{\text{rel}}$. Our experiments also allowed the first comparison of maximum $k_{\text{act}}$ 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.$^{10,11}$ 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 ($\approx 250$ g) were killed directly by cervical dislocation. These procedures were in accordance with UK Home Office guidelines (schedule 1). The hearts were removed, and trabeculae (diameter, 50 to 200 $\mu$m; length, 1.5 to 8 mm) were dissected from the right ventricles in Tyrode’s solution containing 25 mmol/L 2,3-butanedione monoxime to minimize cell contracture. Trabeculae were skinned for 30 minutes in the standard skinned muscle solution (see below) to which was added 1% Triton X-100, 10 mmol/L K$_2$EGTA, 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.

Diazom-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.$^{14}$ Clampfit 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 creatine 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-

ture and calculation of free ion concentrations have been given elsewhere.$^{15,16}$ For determination of the force-pCa relationship and of $k_{\text{rel}}$, solutions also contained 10 mmol/L EGTA and 0 to 10 mmol/L Ca$^{2+}$, giving a pCa (~log$_{10}$[Ca$^{2+}$]) of 9 to 4.5. For the photolysis experiments, solutions were as above but contained 0.25 mmol/L diazo-2 and 0 to 0.25 mmol/L CaCl$_2$ (final pCa $\approx 7.5$ to 4.9), or 2 mmol/L NP-EGTA and 0.6 to 0.8 mmol/L Ca$^{2+}$ (pCa $\approx 7.0$ to 6.0). In the absence of caged compounds, relaxation or maximal Ca$^{2+}$ activation in the photolysis experiments was produced by solutions containing 1 mmol/L EGTA (pCa 8.5) or 1 mmol/L CaEGTA+0.1 mmol/L CaCl$_2$ (pCa 4.5), respectively. Diazom-2 and NP-EGTA (tetrapotassium salts from Molecular Probes) were stored frozen in aqueous stock solutions (25 and 10 mmol/L, respectively); other chemicals were from Sigma or B.D.H.

**Protocols**

As previously described,$^{15}$ each skinned trabecula was first maximally activated several times at 22°C until a reproducible level of force was obtained. For the photolysis parts of the experiment, the muscle was placed in either a diazo-2 solution or an NP-EGTA solution, with total [Ca] adjusted so that the muscle developed 40% to 80% of maximum force in the diazo-2 solution but only a small force (<10% maximum) in the NP-EGTA solution. Once force had stabilized, the flash lamp was triggered, giving a flash of near-UV light (160 mJ in ~1 millisecond) to the muscle. After the force response, each muscle was returned to the relaxing solution. Any gradual decline in maximum force due to deterioration of the muscles was corrected for by assuming a linear decrease between activations. Muscles were discarded when maximum force had fallen by 25%.

$k_{\text{rel}}$ after a rapid release and restretch of the muscle was measured at both 22°C and 12°C. During maximal activation, the muscle length was decreased by 20% in 1 millisecond, held for a 25- or 100-millisecond “holding period” (for rat and guinea pig trabeculae, respectively), and then stretched back to the original length in 1 millisecond. The force redevelopment was recorded. The muscle was subsequently relaxed. The procedure was repeated at various sub-maximal [Ca$^{2+}$] levels, ending with a repeat of the maximal activation. The force-pCa relationship of the trabecula was thereby determined simultaneously with $k_{\text{rel}}$.

In some trabeculae we could record at least 2 of the 3 sets of measurements ($k_{\text{rel}}$, rapid activation, and rapid relaxation) before maximum force declined by 25%.

**Data Analysis**

The relationship between steady-state force and pCa in each muscle was fitted by the Hill equation: relative force=maximum force[$\operatorname{Ca}^{2+}$]n/(K$^{n}+$[Ca$^{2+}$]$^{n}$), where n$_{h}$ is the Hill coefficient and K is a dissociation constant. The changes of force after diazo-2 or NP-EGTA photolysis and the release/restretch protocol were fitted with single-exponential curves (except relaxation in the rat, which was fitted with a double exponential) using Clampfit software (Axon Instruments). Values are given as mean±SEM from n trabeculae (each from a different heart). Unless stated otherwise, unpaired t tests were applied, and a significant difference was taken as P<0.05.

**Results**

Ca$^{2+}$ Sensitivity of Rat and Guinea Pig Myofibrils

In maximal activating solution (pCa 4.5) at 22°C, rat skinned trabeculae (mean diameter, 121±7 $\mu$m; n=45) generated a stress of $78.9±7.8$ mN · mm$^{-2}$, which was not significantly different from that (50.6±8.2 mN · mm$^{-2}$) produced by guinea pig skinned trabeculae (mean diameter, 131±12 $\mu$m; n=9). Resting SL was 2.14±0.01 $\mu$m (n=36) in rat trabeculae and 2.13±0.01 (n=7) in guinea pig trabeculae. The force-pCa relationships for both spe-
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.\textsuperscript{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 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 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).

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 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).
Activation and Relaxation of Cardiac Myofibrils

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 ($k_{\text{rise}}$) 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.

$k_{tr}$ (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 $k_{tr}$ and force was curved upward.

**Rate of Redevelopment of Force**

We investigated whether the difference in $k_{tr}$ between species (Figure 3) was paralleled by a difference in crossbridge kinetics, as measured at constant [Ca$^{2+}$] by $k_{tr}$ 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 $k_{tr}$ 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 $k_{tr}$ 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 $k_{tr}$ 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 $k_{tr}$ between species was not due to the different holding periods used, since $k_{tr}$ 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 $k_{tr}$ recorded from rat trabeculae (Figure 5). The $k_{tr}$ values above were not significantly different from the maximum values of $k_{tr}$ 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 $k_{tr}$ and steady-state force was also determined for each species (Figure 5C and 5F) in order to compare the dependencies of $k_{tr}$ and of $k_{act}$ 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 $k_{tr}$ values at different force levels we pooled data for each muscle. Values of $k_{tr}$ 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 $k_{tr}$ values (relative force, 1.0) in both species. Thus, $k_{tr}$ was independent of force significantly different from the corresponding maximum rates of relaxation.

To determine whether $k_{act}$ 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
below ≈60% of maximum but increased at higher force levels. The data in Figure 5C and 5F were averaged and replotted as $k_{tr}$ against pCa (Figure 6). As $[Ca^{2+}]$ rose from pCa 6.0 to 4.5, $k_{tr}$ 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 $k_{tr}$ value ($\approx 5.4$, Figure 6) was less than the pCa 50 for force (5.6, Figure 1). Thus, force was more sensitive than $k_{tr}$ to $Ca^{2+}$, which may account for the downward curvature in the force-$k_{tr}$ relationships (Figure 5).

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

Brenner and Eisenberg$^{19}$ 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 $k_{tr}$ 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 $k_{tr}$ was still markedly faster in the rat (6.41±0.51 s$^{-1}$, n=8) than in the guinea pig (0.97 s$^{-1}$, n=1). During force redevelopment, SL varied by <3% in both rat and guinea pig trabeculae. Thus, the 6-fold faster $k_{tr}$ 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 $Ca^{2+}$ dissociates from TnC ($k_{off}$) 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 $Ca^{2+}$ is lost from TnC. The identical force-pCa relationships of the 2 species (Figure 1 and Reference 13) imply that $k_{off}$ should be similar in the 2 species. ($Ca^{2+}$ sensitivity is largely determined by the $Ca^{2+}$ affinity constant of TnC, which is equal to the ratio of the on and off rate constants for $Ca^{2+}$ binding: affinity = $k_{on}/k_{off}$. Since $k_{on}$ has been reported to be diffusion-limited$^{20}$ and therefore is constant, affinity may be determined chiefly by $k_{off}$.) Crossbridge kinetics, however, are known to be faster in the rat than in the guinea pig.$^{12,13}$ 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 \(k_{\text{off}}\) but rather by the detachment rate of the crossbridges themselves.

Activation Rate Is Determined by Properties of Crossbridges

Although measurements were made without SL control, and internal shortening could have slowed force development,\(^{19}\) our \(k_v\) 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\(^{8-10}\) when differences in temperature were taken into account. \(k_v\), which reflects the rate of crossbridge reattachment and transition to force-generating states,\(^2\) 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, \(k_{\text{act}}\) is similar to \(k_v\) 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 \(k_{\text{act}}, k_v,\) and the rate of relaxation were \(\approx 5\) 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 ktr, particularly at low [Ca2+]i.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 k0, kact, 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 Ca2+ activation15 and thus would have little effect on the maximum values of k0 and kact, 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).

k0 and kact Are Ca2+ Dependent
The values of k0 and kact increased as Ca2+-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 Ca2+ dependence of k0 in cardiac muscle. Hancock and colleagues7,8 reported no change in k0 when Ca2+ was varied in intact7 and skinned8 cardiac muscles, whereas a Ca2+ dependence of k0 was observed by other groups using rat skinned trabeculae9 and myocytes.10,11 In the present study, we confirm that k0 is increased as [Ca2+]i 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 Ca2+ independence, of k0. Our results (Figure 4) show the importance of defining the optimal holding period for k0 measurements. Although we found that k0 was activated by Ca2+ 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 k0 is greater in fast skeletal than in slow skeletal fibers.6 The significance of the Ca2+ dependence of k0 (and kact) remains to be clarified: as with previous studies, our finding that k0 and kact are Ca2+ dependent could support...
Brenner’s kinetic model, in which Ca\(^{2+}\) regulates force by increasing \(f_{\text{app}}\), or could be the result of cooperative binding of strong crossbridges in the steric blocking model.\(^{6,7}\)

The present report, which represents the first comparison of activation and relaxation rates in the same muscles, shows that the maximum relaxation rate can be as fast as maximum \(k_{\text{on}}\) or \(k_{\text{off}}\). This similarity is difficult to reconcile with any of the models for the Ca\(^{2+}\) dependence of \(k_{\text{a}}\). From the kinetic model,\(^2\) the rate constant of force change after a sudden alteration of [Ca\(^{2+}\)] should equal \(f_{\text{app}}' + g_{\text{app}}\), where \(f_{\text{app}}'\) is the new \(f_{\text{app}}\) at the final [Ca\(^{2+}\)] level, and \(g_{\text{app}}\) is the Ca\(^{2+}\)-independent rate of crossbridge detachment; thus, \(f_{\text{app}}'\) when [Ca\(^{2+}\)] is low after photolysis of diazo-2 will be much smaller than when [Ca\(^{2+}\)] is high after photolysis of NP-EGTA or during maximal \(k_{\text{a}}\) measurements, and the maximum relaxation rate should correspondingly be substantially smaller than the maximum \(k_{\text{on}}\) or \(k_{\text{off}}\). We observed no such difference in rates. Our calculations using Campbell’s cooperative model\(^2\) indicate that relaxation in this model should also be slower than \(k_{\text{on}}\) or \(k_{\text{off}}\) (results not shown). Thus, the rapidity of relaxation remains to be explained. It is unlikely to be due to SL changes during relaxation, since the sarcomeres would relengthen during relaxation and (from the force-velocity relationship) this would tend to maintain force, thereby slowing relaxation. Another possibility is that relaxation involves a reversal of the crossbridge working stroke. It is well established that the force-generating step (which is closely associated with the \(P_{\text{c}}\) release step) is reversible.\(^2\) Neither of the above models includes this concept. We suggest that during relaxation a substantial fraction of crossbridges may lose force by a reversal of the force-generating transition rather than by the slower forward detachment step. The net rate of the backward strong-weak transition is relatively fast (\(\sim 8\) s\(^{-1}\) for rat at 1.7 mmol/L P, and 15°C; estimated from Reference 17), and a contributory loss of crossbridge force via this route could potentially account for the fast rate of relaxation.

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 myofilibrillar 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 \(k_{\text{a}}\) but may be explained if a reversal of the crossbridge working stroke contributes to relaxation.

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