Ca$^{2+}$ regulates the kinetics of tension development in intact cardiac muscle

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trabeculae from rat hearts at varying external Ca$^{2+}$ concentrations ([Ca$^{2+}$]) at 22°C. During tetani, cross bridges were mechanically disrupted and the kinetics of tension redevelopment were assessed from the rate constant of exponential tension redevelopment ($k_{tr}$). There was a relationship between $k_{tr}$ and external [Ca$^{2+}$] that was similar in form to the relationship between tension and [Ca$^{2+}$]. Thus a close relationship existed between $k_{tr}$ and tension ($r = 0.88; P < 0.001$); whereas at maximal tetanic tension (saturating cytosolic [Ca$^{2+}$]), $k_{tr}$ was $16.4 \pm 2.2$ s$^{-1}$ (mean $\pm$ SE, $n = 7$), at zero tension (low cytosolic [Ca$^{2+}$]), $k_{tr}$ extrapolated to 20% of maximum ($3.3 \pm 0.7$ s$^{-1}$). Qualitatively similar results were obtained using different mechanical protocols to disrupt cross bridges. These data demonstrate that tension redevelopment kinetics in intact cardiac muscle are influenced by the level of Ca$^{2+}$ activation. These findings contrast with the findings of one previous study of intact cardiac muscle. Activation dependence of tension development kinetics may play an important role in determining the rate and extent of myocardial tension rise during the cardiac cycle in vivo.

THE GOAL OF THIS STUDY was to determine whether Ca$^{2+}$ regulates the kinetics of tension development in intact cardiac muscle. In contracting skinned skeletal muscle fibers, Brenner (4) demonstrated that after cross bridges were mechanically disrupted, the kinetics of tension redevelopment were faster with increased levels of activation by Ca$^{2+}$. This finding has been confirmed in many recent studies (6, 20–22, 25, 28). However, studies in cardiac muscle have produced markedly differing results. Some studies of both intact (11) and skinned (12) cardiac muscle found that tension redevelopment kinetics were not influenced by Ca$^{2+}$. This raised an important possibility that regulation of contraction by Ca$^{2+}$ in cardiac muscle may be fundamentally different from that in skeletal muscle. In contrast, other studies

in skinned cardiac muscle found that Ca$^{2+}$ does influence tension redevelopment kinetics (31, 33).

Uncertainty on this important aspect of Ca$^{2+}$ regulation of cardiac muscle contraction prompted its reevaluation using intact cardiac muscle in the present study. The issue of whether there is an influence of Ca$^{2+}$ on force redevelopment kinetics may have important physiological implications for contraction of the heart in vivo. Activation-dependent tension-generating kinetics may contribute to determining the rate and extent of myocardial force redevelopment during the cardiac cycle (33).

The experimental approach was to produce steady-state tetanic contractions of intact right ventricular (RV) trabeculae. During tetani, cross bridges were mechanically disrupted using a protocol similar to that used by Brenner (4) in skeletal muscle. Trabeculae were subjected to a brief period of unloaded shortening followed by rapid restretch to initial muscle length (release-restretch protocol). Tension redevelopment kinetics were assessed from the rate constant of tension redevelopment ($k_{tr}$) when cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}$]) was varied by altering superfusate [Ca$^{2+}$]. Previous studies raised the possibility that the time course of tension redevelopment may be influenced by the nature of the length change protocol used to disrupt cross bridges (31, 33). Therefore, we measured $k_{tr}$ using three different mechanical protocols: a release-restretch (4), a sustained release (11), and a brief stretch-release protocol (26).

The results of this study showed that $k_{tr}$ varied fivefold over the full range of Ca$^{2+}$ activation. This demonstrates that regulation of contraction in intact cardiac muscle is similar to that found in skeletal muscle: for both muscle types, the kinetics of the tension-generating processes are influenced by the level of activation.

METHODS

Trabecula preparation. All experiments were performed at 22°C. LBNF1 rats (−350 g, $n = 7$) were anticoagulated with heparin (1,000 U/kg ip) and deeply anesthetized with pentobarbital (85 mg/kg ip). Hearts were removed, placed in a dissection dish, and perfused through the aorta with a modified Krebs-Henseleit solution containing (in mM) 116 NaCl, 5 KCl, 1.2 MgCl$_2$, 1.2 Na$_2$SO$_4$, 2 NaH$_2$PO$_4$, 23 NaHCO$_3$, 10 glucose, and CaCl$_2$ as indicated. Solutions were equilibrated with 95% O$_2$-5% CO$_2$ to obtain a pH of 7.4 at 22°C. To minimize spontaneous contractions during dissection, solutions contained an additional 20 mM KCl and [Ca$^{2+}$] was 0.2 mM.

The RV was opened, and thin unbranched trabeculae running from the RV free wall to the tricuspid valve were isolated as previously described (7). A small cube of the RV...
wall containing a trabecula was removed; a remnant of the valve was left attached. The dimensions of the trabeculae (in mm) were length 2.4 ± 0.3, width 0.25 ± 0.04, and thickness 0.11 ± 0.02 (mean ± SE, n = 7). Trabeculae were mounted in a muscle bath (25 x 3 x 5 mm) and superfused (at ≈5 ml/min) by Krebs-Henseleit solution with 0.5 mM [Ca2⁺].

The cube of ventricle was held in a basket made of platinum wire attached to a strain gauge (model 403; Cambridge Technology, Watertown, MA); the valve remnant was mounted on a stainless steel hook attached to a position-sensitive motor (model 300; Cambridge Technology) (3, 7). To reduce end compliance, we immobilized muscles to their mounts using a method described by Julian et al. (15, 16). Using loose ties of 10-0 suture, we lightly held the ends of the muscles against two small stainless steel pins that emerged axially from the basket and hook mountings. The chamber was briefly drained, and the muscle ends up to the suture were cemented to their mounts using cyanoacrylate tissue adhesive (Histoacryl Blau, Mellsungen, Germany). This method of attachment was shown to markedly reduce end compliance (16).

Muscles were field stimulated using platinum wire electrodes in the side walls of the chamber. Stimulation consisted of 4-ms stimulus pulses; voltage was set to 50% above that giving maximum tension, and frequency was 0.5 Hz. Muscle length was adjusted to maximize the twitch tension. The [Ca2⁺] was gradually raised to 1.2 mM over 15 ms, and stimulated muscles were allowed to equilibrate for 1 h.

Signals from the tension transducer and motor were digitized at 1,000 Hz and stored in a laboratory computer.

Experimental protocols. Superfusate [Ca2⁺] was lowered to 0.25 mM (the first concentration in the range to be studied). To allow steady-state tetani to be induced, we inhibited Ca2⁺ uptake to the sarcoplasmic reticulum (SR) with 100 µM cyclopiazonic acid (CPA) (2, 9). CPA is a specific inhibitor of the SR Ca2⁺-ATPase (30); it does not impair tension development by the myofilaments (17) and does not affect the Ca2⁺ sensitivity of intact RV trabeculae (2). After a 30-min equilibration with the drug, twitch stimulation was interrupted with tetanization at 10 Hz for 7 s. In seven experiments, during the tetanus (after 4 s of stimulation), cross bridges were mechanically disrupted using three different methods that gave similar results. First, cross bridges were disrupted by slackening the muscle by rapidly decreasing the muscle length by 15–20% for 40 ms and then rapidly restretching to the initial length (length changes took 1.5 ms to complete) (n = 4) (4). In one of these four experiments a second protocol was also used consisting of a rapid sustained decrease in muscle length (4% of initial length) that reduced tension close to the baseline (11, 12). Finally, cross bridges were also disrupted by a brief stretch of the muscle (≈8% increase and then decrease in muscle length over 3 ms) (26). For this protocol, the muscle length was first reduced to 92% (n = 3).

To assess the influence of Ca2⁺, the time course of tension redevelopment, we incrementally increased superfusate [Ca2⁺] from 0.25 to 9 mM. After [Ca2⁺] was changed, muscles were equilibrated for 15 min at a stimulation frequency of 0.5 Hz before being tetanized.

Data analysis and statistics. After the length perturbation, the rate constant of monoeponential tension redevelopment (kT) was determined by fitting the rise of tension according to the following relation

\[ f = f_{\text{max}} (1 - e^{-k_T t}) + f_0 \]  

(1)

where f, the force at time t after the length perturbation, rose from f0, the force just after the length perturbation, to a final maximum, \( f_{\text{max}} + f_0 \). Curve fitting was performed using SigmaPlot software (SPSS, Chicago, IL).

Data are reported as means ± SE. Statistical tests and linear regressions were performed using commercial software. Statistical tests were considered significant with P < 0.05.

RESULTS

Tetanization of trabeculae. To induce steady-state contractions in cardiac trabeculae, we tetanized muscles (n = 7) in the presence of CPA (2). Figure 1 shows records of tension during tetanization at different superfusate [Ca2⁺]. Increased superfusate [Ca2⁺] resulted in increased tetanic tension. Figure 2 shows the relationship between tetanic tension and superfusate [Ca2⁺]. Figure 2 shows that the increase of tetanic tension had saturated at high superfusate [Ca2⁺]. The maximum tetanic tension at saturating [Ca2⁺] was 7.8 ± 1.6 g/mm² (mean ± SE, n = 7).

Disruption of cross bridges during tetanization. The records in Fig. 1 also show the effects of mechanically disrupting cross bridges during tetanization using a perturbation of muscle length. This protocol consisted of a release (≈15% muscle length decrease sustained for 40 ms)
followed by restretch to initial length. This protocol caused a substantial reduction of tension. After restretch to the initial muscle length there was a period of tension redevelopment during which tension recovered close to the level before the length perturbation.

Similar behavior was observed using three different protocols to mechanically disrupt cross bridges. The percentage of tetanic tension remaining immediately after the length perturbation was 21 ± 5% (n = 4) for the release-restretch protocol. Lower residual tensions were found using a sustained release (12%) or brief stretch-release protocol (0–2%, n = 3). However, the major findings of this study were the same for all protocols.

Time course of tension redevelopment. To investigate the influence of [Ca\(^{2+}\)] on cross-bridge turnover kinetics, we quantitated the time course of tension redevelopment after length perturbations at different superfusate [Ca\(^{2+}\)] (4). Tension redevelopment records from Fig. 1 are shown with greater temporal resolution in Fig. 3A. The closed circles superimposed on each trace indicate the time at which tension redeveloped to 90% of maximum after the length perturbation. Figure 3A shows that at high superfusate [Ca\(^{2+}\)], tension redeveloped to the 90% level sooner than at low superfusate [Ca\(^{2+}\)]. Thus the time course of tension redevelopment was influenced by Ca\(^{2+}\) and was faster at high superfusate [Ca\(^{2+}\)] compared with low superfusate [Ca\(^{2+}\)].

The influence of [Ca\(^{2+}\)] on tension redevelopment kinetics can be readily appreciated after tension records are normalized to the maximum tension for each contraction (Fig. 3B). As superfusate [Ca\(^{2+}\)] was increased, the time course of tension redevelopment became progressively faster. These data clearly show that, in this study, tension redevelopment kinetics in intact cardiac muscle are influenced by Ca\(^{2+}\).

Effect of Ca\(^{2+}\) on time course of tension redevelopment. The time course of tension redevelopment after the length perturbation was fit to an exponential function (Eq. 1). Figure 3A shows the result of fitting Eq. 1 to the rise of tension at the highest and lowest superfusate [Ca\(^{2+}\)] (dotted lines). Figure 3A shows that the fits coincided closely to the data. The time course of tension redevelopment was quantitated from the rate constant of tension redevelopment (k\(_{tr}\)) determined from the fits.

Figure 4 shows the relationship between k\(_{tr}\) and superfusate [Ca\(^{2+}\)] for a subgroup of experiments where cross bridges were disrupted by a brief period of unloaded shortening followed by restretch (n = 4). For each experiment, k\(_{tr}\) values were normalized to the maximum value (the maximum k\(_{tr}\) at the highest superfusate [Ca\(^{2+}\)] was 14.3 ± 2 s\(^{-1}\), mean ± SE, n = 4). Figure 4 shows that for increases of superfusate [Ca\(^{2+}\)] up to 2 mM there was a steep increase of k\(_{tr}\). As superfusate [Ca\(^{2+}\)] was increased beyond 2 mM, there was a shallower rise of k\(_{tr}\). The relationship between k\(_{tr}\) and superfusate [Ca\(^{2+}\)] suggests that increases of cytosolic [Ca\(^{2+}\)] are associated with faster tension redevelopment kinetics. The form of the saturating relationship between k\(_{tr}\) and superfusate [Ca\(^{2+}\)] is similar to the saturating relationship between tension and superfusate [Ca\(^{2+}\)] shown in Fig. 2. This suggests that increases superfusate [Ca\(^{2+}\)] causes both a rise of tension and faster tension redevelopment kinetics.

To further assess the relationship between the kinetics of tension redevelopment and the extent of cardiac muscle activation, Fig. 5 shows the relationship between k\(_{tr}\) and tetanic tension (this relationship equates those shown in Figs. 2 and 4). For each experiment,
values of $k_{tr}$ and tension were normalized to their maximum tetanic levels. Figure 5 shows there was a close positive relationship between $k_{tr}$ and tension: a linear regression fit to the data (not shown) was statistically significant ($r = 0.92$, $P < 0.001$). This close relationship between $k_{tr}$ and tension further suggests that the kinetics of tension redevelopment are strongly influenced by the degree to which cardiac muscle is activated by $Ca^{2+}$.

We also investigated whether the close relationship between $k_{tr}$ and tension shown in Fig. 5 was influenced by end compliance. Muscle end compliance is a concern because sarcomere lengths were not controlled. For each experiment, the slope of the relationship between relative $k_{tr}$ and tension was plotted against the respective muscle length (Fig. 5, inset). If end compliance were an important determinant of the relationship between $k_{tr}$ and tension, then the influence of end compliance should be reduced for longer muscles. In contrast, the data show that the slope of the relationship between relative $k_{tr}$ and relative tension was not significantly related to muscle length ($r = -0.069$, $P = 0.93$). This suggests that the influence of end compliance due to lack of length control was not responsible for the close relationship observed between $k_{tr}$ and tension. This simple analysis is quantitatively limited by the apparent nonlinearity of the relationship between relative $k_{tr}$ and relative tension. However, it does show qualitatively that the relationship found between relative $k_{tr}$ and relative tension is not greatly influenced by muscle length.

Measurement of $k_{tr}$ using different protocols to disrupt cross bridges. Previous studies have used different length change protocols to disrupt cross bridges. Use of different length change protocols has been suggested as a factor that may have contributed to conflicting results regarding the dependence of $k_{tr}$ on $Ca^{2+}$ in cardiac muscle (31, 33). Therefore, we confirmed our findings using two additional protocols to disrupt cross bridges. In one of the experiments described above, cross bridges were also disrupted by a rapid and sustained decrease in muscle length (release) (11, 12). In a third protocol, muscles were briefly and rapidly stretched to forcibly detach cross bridges (stretch-release protocol) ($n = 3$) (26). Examples of tension transients after these protocols are shown in Fig. 6. Figure 6, bottom, shows tension records normalized to the maximum values attained after the mechanical perturbations. The normalized data clearly show that, after cross bridges were disrupted by a release (Fig. 6A) or stretch-release protocol (Fig. 6B), the time course of tension redevelopment was faster under conditions of high extracellular $Ca^{2+}$. This is consistent with the findings shown in Fig. 3 using the release-restretch protocol.

Figure 6, bottom, also shows the results of fitting Eq. 1 to the redevelopment of tension. At both high and low $[Ca^{2+}]$, the fitted curves (dotted lines) almost superimpose on the data (solid lines), demonstrating that tension redevelopment was close to exponential. At maximum extracellular $[Ca^{2+}]$, the value of $k_{tr}$, obtained using the stretch-release protocol (19.3 ± 4.5 s⁻¹, $n = 3$) was similar to that found using the release-restretch protocol ($P = 0.3$). However, the value of $k_{tr}$ at maximum extracellular $[Ca^{2+}]$ obtained using the release protocol (43 s⁻¹, $n = 1$) appeared higher. This is consistent with a previous study that found that $k_{tr}$ for high superfusate $[Ca^{2+}]$. 

![Fig. 5](http://ajpheart.physiology.org/)

**Fig. 5.** Relationship between $k_{tr}$ after a release-restretch protocol vs. tetanic tension (where tension was varied by varying superfusate $[Ca^{2+}]$). Data were normalized to maximum values for each experiment (means ± SE; $n = 4$). Inset: slope of relationship between $k_{tr}$ and tension for each experiment plotted vs. muscle length for each experiment.

![Fig. 6](http://ajpheart.physiology.org/)

**Fig. 6.** A: time course of tension redevelopment after a release to disrupt cross bridges. B: time course of tension redevelopment after a stretch-release protocol to disrupt cross bridges. A and B: top, change in muscle length; middle, tension vs. time; bottom, tension normalized to maximum attained after length change. Normalized tension redevelopment data at high and low superfusate $[Ca^{2+}]$ were fit to Eq. 1 (dotted lines); fitted curves almost superimpose onto data. Tension redevelopment was exponential and was faster with high superfusate $[Ca^{2+}]$. 

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measured after a release was approximately twice that measured after a release-restretch protocol (12).

Data using all three mechanical protocols from all experiments are summarized in Fig. 7. Figure 7 shows that, taken together, data for all three protocols formed a close relationship between $k_r$ and tension. A linear regression fit to all of the data of this study (solid line) showed a significant positive correlation between $k_r$ and tension ($r = 0.88$, $P < 0.001$). For all experiments, at maximal tetanic tension (saturating cytosolic [Ca$^{2+}$]$_i$), $k_r$ was $16.4 \pm 2.2$ s$^{-1}$ (mean $\pm$ SE, $n = 7$). At zero tension (low cytosolic [Ca$^{2+}$]$_i$) $k_r$ extrapolated to 20% of maximum ($3.3 \pm 0.7$ s$^{-1}$). These data demonstrate that in intact cardiac muscle there is activation dependence of tension redevelopment kinetics. Furthermore, this finding does not depend on the use of a particular protocol to disrupt cross bridges.

**DISCUSSION**

The major finding of this study was that after mechanical disruption of cross bridges during tetanization of intact cardiac muscle, the kinetics of tension redevelopment were faster when superfusate [Ca$^{2+}$]$_i$ was elevated. This finding demonstrates that in intact cardiac muscle, Ca$^{2+}$ influences the kinetics of the tension-generating process. This finding is consistent with results from studies of skinned skeletal (4, 6, 20, 21, 25, 28) and cardiac muscle (31, 33) but differs from the results of other studies of intact and skinned cardiac muscle (11, 12).

The physiological significance of this finding is that the influence of Ca$^{2+}$ on the kinetics of the tension-generating processes may contribute to determining the rate and extent of tension development during the cardiac cycle.

Effect of Ca$^{2+}$ on the kinetics of tension generation. Previous studies in skeletal muscle found that when cross bridges were mechanically disrupted by a brief period of unloaded shortening followed by restretch, the rate constant of tension redevelopment ($k_r$) was increased from 5- to 10-fold when [Ca$^{2+}$]$_i$ was raised from low to saturating levels (4, 6, 20, 21, 25, 28).

Findings in cardiac muscle have been controversial. Studies using skinned cardiac muscle also found that $k_r$ was increased with [Ca$^{2+}$] (31, 33). In contrast, other studies in intact and skinned cardiac muscle found that $k_r$ was not influenced by Ca$^{2+}$ (11, 12). These studies raised the possibility that cardiac muscle may be fundamentally different from skeletal muscle in terms of how tension is regulated by Ca$^{2+}$.

The present study has addressed the conflicting findings from these previous reports. We used thin intact rat RV trabeculae that are metabolically stable at high work loads (29), develop stable steady-state tensions during tetanization (2), and had low residual tensions when cross bridges were disrupted with mechanical protocols.

We found that, similar to skeletal muscle, in intact cardiac muscle Ca$^{2+}$ does increase the kinetics of tension redevelopment after cross bridges are mechanically disrupted by a rapid decrease of muscle length followed by restretch.

Some of the disparity in previous reports was suggested to arise from differences in the mechanical protocols used to disrupt cross bridges (31, 33). For example, when cross bridges are disrupted by a rapid decrease of muscle length (i.e., without restretch), even though tension declines to zero, cross bridges may remain attached to actin. The presence of a residual fraction of attached cross bridges may influence the level of activation and thus could mask the influence of Ca$^{2+}$ on $k_r$. Therefore, in the present study we monitored $k_r$ using several different protocols to disrupt cross bridges. We found there was a similar activation dependence of tension redevelopment kinetics when cross bridges were disrupted using a release-restretch protocol, a release (without restretch), or a brief stretch-release protocol. This suggests that the finding of an influence of Ca$^{2+}$ on $k_r$ does not depend on the use of a particular mechanical protocol to disrupt cross bridges.

Role of Ca$^{2+}$ in activation of cardiac muscle contraction: relation to $k_r$. Classically, Ca$^{2+}$ has been thought to participate in muscle activation by binding onto troponin and causing a structural change in tropomyosin, which then allows myosin cross bridges to undergo cyclic interactions with actin (14, 19, 24, 32). Thus Ca$^{2+}$ was thought to cause muscle activation by causing increased numbers of cross bridges to cycle with fixed turnover kinetics between attached high-force states and detached low-force states (27). According to this simple recruitment scheme, $k_r$ would not be expected to vary with [Ca$^{2+}$].

However, this recruitment hypothesis was brought into question by Brenner (4), who found that $k_r$ was influenced by [Ca$^{2+}$] in skinned skeletal muscle fibers. This finding was interpreted within the framework of a simple two-state cross-bridge model based on that described by Huxley (13). According to this simple model, $k_r$ reflects cross-bridge turnover kinetics. Thus the finding that $k_r$ depends on [Ca$^{2+}$] suggested that in skeletal muscle Ca$^{2+}$ activates contraction by regulat-
ing cross-bridge turnover kinetics rather than recruiting cross bridges to cycle.

However, the mechanism by which \([\text{Ca}^{2+}]\) determines \(k_r\) remains uncertain. More recent studies suggest that \(\text{Ca}^{2+}\) influences \(k_r\) by interaction of \(\text{Ca}^{2+}\) with regulatory light chains (22, 25) or troponin C (6, 28) and through dynamic modulation of thin-filament regulatory units (6). Recent mathematical models suggest that the \(\text{Ca}^{2+}\) dependence of \(k_r\) may involve cooperativity between force-bearing cross bridges and the level of activation (5), or coupling between the two processes of \(\text{Ca}^{2+}\) binding to the thin filament and force generation (10, 18).

Despite this uncertainty regarding mechanism, the finding that \(k_r\) is activation dependent in intact cardiac muscle likely has important physiological significance. Thus the broader context of our finding is in terms of a role for \(\text{Ca}^{2+}\) in regulating the rate and extent of tension rise during the cardiac cycle (33).

Relation to previous studies. There has been only one previous study of \(k_r\) in intact cardiac muscle (11). In marked contrast to the findings of the present study, the previous study found that the kinetics of tension redevelopment were not influenced by the level of activation (11). Several differences in experimental design between these two studies should be considered. First, the present study used rat myocardium, whereas Hancock et al. (11) used ferret myocardium. However, Hancock et al. (12) using skinned rat myocardium also found \(k_r\) was not activation dependent. This raises the possibility that some other difference besides species may explain the conflicting findings.

Second, the present study was performed at 22°C, whereas Hancock et al. (11) used 27°C. In a preliminary report, de Tombe and Steinen (8) found greater activation dependence of \(k_r\) at higher temperature. This suggests that the small temperature difference between the present and previous study of intact myocardium cannot explain the different findings.

Third, the present study used CPA to perform tetanization, whereas Hancock et al. (11) used ryanodine. Previous studies found similar tetanic force and cytosolic \(\text{Ca}^{2+}\) levels using tetanization with CPA or ryanodine (2). Furthermore, CPA did not affect the function of the contractile proteins (17, 30). These findings suggest that the different pharmacological methods used to tetanize do not account for the different findings between the present study and that of Hancock et al. (11).

Fourth, Hancock et al. (11) used feedback control to maintain a central segment of muscle at a constant length. The present study did not use length control. Length control minimizes the shortening of central sarcomeres because of end compliance. End compliance could lead to underestimation of \(k_r\) (6, 33). However, in the present study, there was similar activation dependence of \(k_r\) in both short and long muscles, even though shorter muscles should be more susceptible to the effects of end compliance. This suggests that problems of end compliance due to lack of length control do not underlie our finding that \(k_r\) was activation dependent. This conclusion is consistent with a study by Wolff et al. (33) that noted the relationship between \(k_r\) and tension in skinned rat myocardium was similar both with and without sarcomere length control (although a detailed comparison was not presented).

Finally, Hancock et al. (11) used a release to mechanically disrupt cross bridges. The present study used three different mechanical protocols to disrupt cross bridges and found similar activation dependence with all three methods. This suggests that in the present study, the finding that \(k_r\) was activation dependent does not depend on the use of a particular protocol to disrupt cross bridges.

In summary, despite some differences in experimental design, the differing results of the present and previous study of intact cardiac muscle cannot be reconciled. Thus the present study extends the controversy concerning the \(\text{Ca}^{2+}\) dependence of \(k_r\) to an intact cardiac muscle preparation. However, the present study is consistent with growing evidence that \(\text{Ca}^{2+}\) does play a role in modulating the kinetics of tension redevelopment in cardiac muscle. The present results are consistent with studies of skinned cardiac trabeculae (8, 33) and skinned cardiac myocytes (31). Furthermore, when skinned trabeculae were activated to different levels by liberating \(\text{Ca}^{2+}\) from a photolabile chelator, then the rate constant for tension development was increased at higher levels of activation (1, 23).

In summary, the present study suggests that, qualitatively, cardiac and skeletal muscle are similar in that both display activation-dependent tension redevelopment kinetics.

Limitations of study. In the present study, sarcomere lengths were not controlled. However, we found the activation dependence of \(k_r\) was not influenced by muscle length. As discussed above, this suggests that problems of end compliance due to lack of length control do not account for our finding that \(k_r\) was activation dependent. Future studies of intact cardiac muscle using length control would be valuable.

Incomplete cross-bridge detachment could lead to further underestimation of the influence of \(\text{Ca}^{2+}\) on \(k_r\) (31, 33). We used three different protocols to disrupt cross bridges. These protocols resulted in a low residual tension, ranging from ~20% using a release-restretch protocol to almost none (~2%) using a brief stretch-release protocol. However, similar results were obtained using each protocol. This suggests that the overall finding of this study is not altered by the presence of small residual tensions after disrupting cross bridges.

In conclusion, this study demonstrates that, in contracting intact cardiac muscle after cross bridges are disrupted, the kinetics of tension redevelopment are faster at higher levels of activation. This finding is similar to the behavior observed in skeletal muscle and suggests that for both muscle types activation of contraction by \(\text{Ca}^{2+}\) involves an effect on tension-generating kinetics. This conclusion has important implications for myocardial tension development during the cardiac cycle in vivo.
REFERENCES


