Deactivation of Contraction by Quick Releases in the Isolated Papillary Muscle of the Cat

EFFECTS OF LEVER DAMPING, CAFFEINE, AND TETANIZATION

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ABSTRACT

In the isolated cat papillary muscle, the time course of activation during isometric contraction was measured using instantaneous velocity of shortening after quick releases at constant contractile element length and controlled load. Activation rose and declined ahead of isometric force. This course of activation could be altered by the mode of the quick release. Undamped quick releases increasingly deactivated contraction with time. Deactivation was reduced by damping the rapid movement of the lever after the release and by increasing the afterloads to which the releases were made, thus lessening the extent of the releases. The curves of activation obtained from releases to different afterloads converged after the first half of the rising phase of the twitch. Deactivation from quick releases was eliminated by tetanizing the muscle. Hence, the instantaneous relation of force and velocity of shortening was not unique relative to time except when the muscle was tetanized. Deactivation was also reduced by lowering the temperature, by adding 10 mM caffeine, or by increasing the Ca²⁺ concentration in the bathing medium in the presence of 10 mM caffeine. These results suggest that quick releases deactivate contraction by uncoupling crossbridges which can be reformed if the Ca²⁺ available to activate contractile sites is not sequestered elsewhere.

KEY WORDS  
activation of contraction  
heart muscle  
calcium  
sarcoplasmic reticulum  
instantaneous force-velocity relation

In A. V. Hill’s model of contracting muscle (1), an active contractile element (CE) is arranged in series with a passive series elastic element (SE). During isometric contraction, the CE shortens and stretches the SE, leading to an increase in force. When the muscle is released to a small load, two phases of shortening are observed: (a) a very rapid shortening reflecting primarily the passive recoil of the SE and (b) a slower sustained shortening reflecting the subsequent active shortening of the CE with a load (1). Quick releases were originally used in skeletal muscle to discharge the SE and study the relation between velocity and load at known CE lengths and times during contraction (2).

In cardiac muscle, the quick-release technique has been widely used to define the intensity and the course of activation of the CE, usually termed active state, by measuring the instantaneous velocity of shortening at constant load and length at any given time in contraction (3–9). This technique has also been used to determine instantaneous force-velocity curves at a constant time in contraction (3–5, 8, 10). Indeed, these curves have been recommended as specific indexes of myocardial contractility (11, 12). However, several studies (4–7, 13, 14) have cautioned that such curves may be subject to a systematic error due to deactivation brought about by the quick release. Also, it has recently been claimed that deactivation can be totally prevented by damping the movement of the lever (15). Nevertheless, the mechanism by which quick releases deactivate contraction is unknown. It has been hypothesized that quick changes in length uncouple crossbridges (7).

In the present study, the effects on activation of contraction of various mechanical alterations in the mode of release and of factors that alter myoplasmic Ca²⁺ concentration were explored. Activation was defined as instantaneous velocity of shortening at constant CE length after quick releases to a given load. The course of activation of contraction was obtained by using these velocities...
of shortening relative to time after quick releases at multiple times after stimulation.

**Methods**

Twenty papillary muscles were removed from the right ventricle of adult cats anesthetized with sodium pentobarbital (25 mg/kg, ip). The cross-sectional area of these muscles ranged from 0.34 mm$^2$ to 1.45 mm$^2$ with an average of 1.09 ± 0.06 mm$^2$ (SE). The muscles were mounted vertically in a bath containing a modified Krebs-bicarbonate solution with the following millimolar composition: NaCl 118, KCl 4.7, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.1, NaHCO$_3$ 24, CaCl$_2$ 2.5, and glucose 5.0. The solution was bubbled with a mixture of 95% O$_2$-5% CO$_2$ and was maintained at either room temperature (22–24°C) or 30°C at pH 7.4. The muscles were stimulated at a rate of 6/min or 12/min with 5-msec rectangular pulses about 10% above threshold. The stimulus was provided through two platinum electrodes arranged longitudinally along both sides of the muscle. With caffeine (10 mM) and high calcium (7.5 mM) in the bathing medium, the stimulation rate was reduced to 6/min at room temperature, because fusion can occur at higher frequencies. Tetani were obtained by delivering 3-second trains of repetitive pulses at a frequency of 10/sec as described previously (16).

The lower nontendinous end of the muscle was held by a spring-loaded Lucite clip, which formed the end of a steel rod that penetrated the bottom of the bath, and was directly attached to a Statham C1-1.5-300 force transducer. The tendinous end of the muscle was held by a light stainless steel clip (mass 50 mg) that was extended upwards via a thin nonmagnetic straightened tantalum wire (mass 40 mg) to the tip of the lever of the electromagnetic lever system, which was mounted on a Palmer stand. The load-extension curve of the total equipment excluding the muscle was exponential, like an electromechanical lever system, which was mounted vertically in a bath containing a modified Krebs-bicarbonate solution with the following millimolar composition: NaCl 118, KCl 4.7, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.1, NaHCO$_3$ 24, CaCl$_2$ 2.5, and glucose 5.0. The solution was bubbled with a mixture of 95% O$_2$-5% CO$_2$ and was maintained at either room temperature (22–24°C) or 30°C at pH 7.4. The muscles were stimulated at a rate of 6/min or 12/min with 5-msec rectangular pulses about 10% above threshold. The stimulus was provided through two platinum electrodes arranged longitudinally along both sides of the muscle. With caffeine (10 mM) and high calcium (7.5 mM) in the bathing medium, the stimulation rate was reduced to 6/min at room temperature, because fusion can occur at higher frequencies. Tetani were obtained by delivering 3-second trains of repetitive pulses at a frequency of 10/sec as described previously (16).

In all experiments the preload on the muscle was adjusted so that the initial muscle length corresponded to Lmax, the length at which actively developed tension is maximum. Resting tension averaged 8.85% of total tension; average actively developed tension and average resting tension were 5.65 ± 0.45 g/mm$^2$ (SE) and 0.5 ± 0.03 g/mm$^2$, respectively, at room temperature.

**ELECTROMAGNETIC LEVER SYSTEM**

The lever system described previously (17) was designed to measure muscle shortening and to impose force on the muscle. The lever (30 mm long) was fashioned from magnesium and attached by cement to a coil that was suspended in the field of a permanent magnet (magnetic induction = 1.2 weber/m$^2$). The total equivalent moving mass of the lever, the coil, and the connections excluding the muscle clip was 225 mg. The equivalent mass of the lever itself was 40 mg. The current through the coil was controlled by two independent transistorized current sources that were allowed to hold the lever and hence the muscle against the upper micrometer stop screw with forces in steps of 0.1, 1.0, and 10.0 g to a total of 19.9 g. Displacement of the lever was measured with a photoelectric transducer, which was linear over a range of 2 mm. Velocity of displacement of the lever and of muscle shortening was derived from an active differentiator. The relation of the highest frequency component in the input signal of the differentiator to its critical frequency was about 0.02.

**RECORDING SYSTEM**

The stimulus, force, shortening, and velocity of shortening were simultaneously displayed relative to time on a storage oscilloscope (Tektronix 564B) and a multichannel oscillograph. Velocity of shortening and force were also recorded in relation to length in the phase plane on a second oscilloscope (Tektronix 5103). Photographs were taken with a Polaroid camera.

A three-component muscle model that included a CE, a SE, and a parallel elastic element (PE) was assumed. The PE was neglected, because the preload was always relatively small, averaging 8.85% of the total developed tension. At these resting tensions, the load shifted from the PE to the CE during a quick release is quantitatively negligible (5, 8). Thus the rapid shortening after the quick release of the isometrically contracting muscle reflects the amount of stretch of the SE at that time and the internal shortening of the CE before the release. The subsequent slower velocity of shortening reflects the CE shortening after the release. In a particular twitch, this velocity of shortening was measured at the same CE length after damped and undamped releases of the isometric contraction to the same isotonic load at multiple times after stimulation (8). The reference length selected for all measurements at different times during a contraction corresponded to that to which the CE had shortened 40–50 msec after a release made at the peak of the isometric force. This reference length ensured that a common CE length (L/ Lmax) would be obtained at which all velocity measurements could be made. The course of activation was obtained by plotting these instantaneous velocities relative to time after stimulation for releases to preload and various afterloads.

**DAMPING OF THE LEVER MOVEMENT**

The undamped quick release was modified by two modes of damping to slow the rapid passive length change resulting from the recoil of the SE and to reduce the subsequent inertial oscillations of the lever. The air jet system described previously (3) was used for damping. The lever was held against the upper stop by an air jet directed against the base of the lever. The muscle was released to a load set by one current source by triggering a solenoid-controlled valve at various delays, which directed the air jet away from the lever. The lever was then free to move, leading to the quick release. The time required to direct the air totally away depended on the capacitance and the elasticity of the tubing connecting the valve with the jet; to achieve damping, it was adjusted to 10–15 msec. Thus, the
passive length change after the release was slowed down, abolishing the initial overshoot of shortening and reducing the inertial oscillations (Fig. 1, part 1). In Figure 1 (part 2) an electronically damped release is compared with an undamped quick release occurring at the same time. Electronic damping was achieved by feeding back the output voltage of an active differentiator of the displacement (dl/dt) of the lever to the coil. Accordingly an afterload (Fig. 1, part 2, center trace) proportional to dl/dt was imposed on the shortening muscle after the release. The damping afterload was not higher than 4 mg/mm sec\(^{-1}\) and was greatest immediately after the release, since dl/dt of the passive rapid shortening of the SE was much higher than that of the active shortening of the CE. By this mode of damping the muscle was slowly released to the final afterload; thus, this mode is called the slow release. At the time the muscle reached the final afterload, the CE had also shortened so that the shortening of the SE and that of the CE were added. The electronic damping of the lever was adjusted to be sufficient for the greatest releases made from the peak of the isometric twitch to avoid overshoots and subsequent oscillations in shortening; it was then held constant for all releases throughout the contraction to ensure comparable conditions. After undamped quick releases, the velocity of shortening and the total amount of shortening were reduced compared with these parameters following damped and slow releases (Fig. 1). The reduction in the velocity and the amount of shortening after an undamped quick release is considered to reflect deactivation of contraction.

### Effects of Mechanical Alterations of the Release on Activation

Figure 2 shows the isometric force and the corresponding course of instantaneous velocity of shortening relative to time after stimulation following undamped, damped quick, and slow releases to preload, 0.5-g afterload, and 1.0-g afterload. The results are representative of those found in eight muscles. Maximum activation represented by velocity of shortening corrected for length after the quick release to the same load is attained early in the first half of the rising phase of the twitch (Fig. 2). The velocities after undamped releases are always the lowest. When the rapid initial movement of the lever is damped by an air jet, higher velocities are seen earlier in contraction, although the curves fall as time progresses. Slow releases are accompanied by lower velocities early in contraction and a sustained curve later in time. When the muscle is released to afterloads of 0.5 g and 1.0 g, the velocity of shortening early in contraction decreases for all modes of release in accordance with the inverse relation of load and velocity of shortening. Later the inverse relation between velocity and load is less apparent. Thus velocity of shortening is determined by the load imposed on the muscle and by the extent of deactivation due to the release to this load. The effects of the slow release later in the contraction cannot be explained merely by the damping of the afterload, because the velocity is always higher than it is after damped and undamped quick releases to 0.5-g and 1.0-g afterloads. Thus the appropriate damping has to be adjusted for each release to any load at any given time. The course of activation in this contraction.

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**Figure 1**

Effects of damping the movement of the lever on velocity of shortening after quick releases. 1: Release is damped by an air jet system. 2: Movement of the lever is damped electronically by feeding back the velocity of shortening signal to the coil of the lever (note the exponentially decreasing afterload following the release in the center trace). Top traces = shortening, center traces = force, and bottom traces = velocity of shortening relative to time. Velocity and total amount of shortening are higher following the damped release; overshoot in shortening and inertial oscillations are reduced. The same lengths are attained at different times (solid vertical lines) following undamped and damped releases occurring at the same time after stimulus (arrows). M.L. = muscle length.
Even with the slow release, it is not possible to avoid deactivation after releases to small loads. In Figure 3 the time course of activation is obtained from undamped and slow releases to various loads. After undamped releases, the velocities converge to a single level 50 msec before the peak of the twitch despite different loads; after slow releases, the curves also converge with increasing time after stimulation. Thus velocity of shortening at 1.0-g and 6.0-g afterload is only slightly different at the peak of the twitch. The inverse relation of force and velocity of shortening, apparent early in contraction, vanishes progressively with time.

From these experiments it is apparent that the time courses of activation and isometric force are dissociated, that the deactivation after quick release may not be prevented by damping alone at times after the peak of contraction, and that, for all modes of release, a smaller extent of release to higher loads induces less deactivation.
EFFECTS OF CHANGE IN TEMPERATURE, CAFFEINE, AND TETANUS ON DEACTIVATION

If the assumptions that quick releases uncouple crossbridges and that these bridges are not reformed in contraction later in time when the Ca²⁺ concentration at contractile sites is lowered due to the uptake of Ca²⁺ by the sarcoplasmic reticulum hold, then deactivation should also be reduced if the sequestration of Ca²⁺ is decreased.

The sequestration of Ca²⁺ by the sarcoplasmic reticulum is reduced in skeletal (18–20) and heart muscle (A. Stam, personal communication) by lowering the temperature of the incubation medium. Accordingly, the course of activation was measured with undamped and damped quick releases at 30° and 24°C and plotted along with the isometric force relative to time after stimulation (Fig. 4). The results are typical of those obtained in five muscles. Deactivation was greater at the higher temperature as reflected by the difference in the velocities of shortening obtained after undamped and damped quick releases. For example, 300 msec after stimulation, the velocity of shortening after an undamped release was 75% at 30°C and 94% at 24°C of the velocity measured with a damped release. The difference between the curves of activation decreased at both temperatures at times after the peak of contraction.

It has been postulated (9) that in heart muscle caffeine increases the amount of Ca²⁺ available at contractile sites by inhibiting the Ca²⁺ uptake by the sarcoplasmic reticulum and increasing the transsarcolemmal flux of Ca²⁺. Therefore, caffeine (10 mM) was added to the bathing medium, and the Ca²⁺ concentration was increased to 7.5 mM. An experiment in one representative muscle is shown in Figure 5, in which shortening (ΔL) and its first derivative (dl/dt) after undamped (note oscillations in the ΔL and dl/dt traces) and slow releases to preload are superimposed and displayed versus time in part 1 and in the phase plane in part 2 of A–D. With caffeine (10 mM) and Ca²⁺ (7.5 mM) (Fig. 5B), dl/dt at any length was greater and the extent of shortening was increased compared with those of control muscles (Fig. 5A). Displaying these parameters relative to time may be misleading, because dl/dt appears to be less and the recoil of the SE is greater after the undamped release. However, at the same length in the phase plane, the velocity of shortening (dl/dt) is the same after both modes of release occurring before the peak of the isometric twitch (Fig. 5A and B). In Figures 5C and D, the releases have been performed after the peak of the twitch. In the control, the undamped release (Fig. 5C) deactivated contraction considerably more than did the slow release; the amount and the velocity of shortening were markedly reduced after the undamped quick release. With caffeine (10 mM) and Ca²⁺ (7.5 mM) (Fig. 5D), the subsequent shortening and velocity of shortening after the undamped release were only slightly smaller than they were after the slower release. Therefore, deactivation was almost prevented. Because of large oscillations, the velocity of shortening following undamped releases cannot be measured at long lengths (Fig. 5D).

When the muscle was tetanized by repetitive stimulation in the presence of 10 mM caffeine and 7.5 mM Ca²⁺, deactivation was inhibited (Fig. 6).
Effects of caffeine (10 mM) and increased Ca\textsuperscript{2+} (7.5 mM) on velocity of shortening (dl/dt) after quick (QR) and slow releases (SLR) during the rising (A, B) and the relaxation phase (C, D) of contraction. In each set (A-D) the subsequent shortening (\(dL\)) and velocity of shortening are superimposed on a storage scope following quick releases (oscillations) and slow releases. These curves are simultaneously displayed versus time (1) (the beam of the scope is triggered by the releases), and they are displayed together with active tension in the phase plane (2). Velocity of shortening at the same length and shortening is the same after quick releases and slow releases occurring in the rising phase of the control contraction (A2). When displayed versus time (A1), the velocity of shortening after a quick release seems to be lower than that after a slow release at the same time. In the relaxation phase, the subsequent amount and velocity of shortening are reduced following quick release in the control contraction (C). In the presence of caffeine (10 mM) and increased Ca\textsuperscript{2+} concentration (7.5 mM), velocity of shortening and shortening following releases in the rising phase of contraction (B) are much greater than those in the control muscles (A). In the relaxation phase (D), velocity of shortening and shortening are little altered following a quick release. Due to the large oscillations after a quick release, velocity of shortening is uncertain at longer lengths (B2, D2). Temperature \(= 24.5^\circ\text{C}\); muscle length \(= 8.9\text{ mm}\), cross-sectional area \(= 1.45\text{ mm}^2\). Control: rate \(= 12\text{/min}\), time to peak force (TPF) \(= 600\text{ msec}\), latent period (LP) \(= 40\text{ msec}\); in the presence of 10 mM caffeine and 7.5 mM Ca\textsuperscript{2+}: rate \(= 6\text{/min}\), TPF \(= 1,000\text{ msec}\), LP \(= 80\text{ msec}\). The traces are redrawn from the original records.

The arrows indicated when the releases occurred in the tetanus (bottom); the releases are displayed in A and B in the same way as described for Figure 5. There was no difference in the velocity of shortening after either undamped quick or slow releases. Thus deactivation was prevented 1,000 msec after the first and the last stimulus, although the intensity of activation had slightly decreased to 80% of the steady-state value 1,000 msec after the last stimulus. After undamped quick releases, velocity of shortening was underestimated and deactivation was overestimated, if displayed versus time (Fig. 6B, part 1). When displayed at corrected lengths in the phase planes (Fig. 6B, part 2), velocities of shortening were not different. This finding stresses the importance of length corrections in comparing the effects of different techniques of release.

The data shown in Figure 7 were obtained from one muscle but are representative of the data for eight muscles and recapitulate some of the results reported above. The course of activation and isometric force was determined relative to time after stimulation. The control value and the effects of 10 mM caffeine are shown in Figure 7 (top). In the bottom of Figure 7, 10 mM caffeine and 7.5 mM Ca\textsuperscript{2+} were present for both the twitch and the tetanic contraction. In the control contraction, the onset of activation was early and fell to 50% of the maximum by the time of peak tension. The deactivation produced by quick releases relative to slow releases was evident. Adding 10 mM caffeine led to an increase in developed force, a prolongation of the latent period between the stimulus and the start of force development, and a delay in the time to peak force, although the decline in isometric force was slowed. Activation was delayed in onset and prolonged; it then declined nearly in parallel with isometric force. Maximum activation was reached at 72.5% of time to peak tension (control 44.5%), and at the peak of the twitch activation was still 88% of maximum. Moreover, velocities of shortening derived from undamped quick and slow releases approached one another. These findings were more pronounced when the Ca\textsuperscript{2+} concentration was increased to 7.5 mM (Fig. 7, bottom). Maximum activation was attained at 72% of time to peak tension, where it was 92% of the maximum. The delayed onset of force development and activation due to caffeine were slightly reversed by increasing the Ca\textsuperscript{2+} concentration. In tetanus, a steady state of activation was achieved for about 2,000 msec; before the plateau of activation was
reached, a transient decrease of activation occurred shortly after the isometric force rose with a slight inflection at the same time above the single twitch. This sequence of events was found in all eight muscles that were tetanized. In tetanus, activation rose and declined in parallel with the isometric force, and deactivation was completely inhibited.

In three additional experiments, isometric force redevelopment was measured after quick releases. The muscle was released to preload for 50 msec without damping at various times after stimulation and then stretched back to the original length; the redeveloped force was measured (Fig. 8). In the control contraction (Fig. 8A), the muscle did not redevelop force after releases from the peak of the isometric twitch. In the presence of 10 mM caffeine (Fig 8B), force was redeveloped in the relaxation phase of contraction. Tension redevelopement was further increased and seen later in the relaxation phase of contraction when the calcium concentration was raised to 7.5 mM (Fig. 8C). Thus, under these conditions, the capacity to redevelop force and to shorten was preserved later into contraction, despite rapid length changes.

Discussion

The present study supports the hypothesis that quick releases of contracting cardiac muscle can produce deactivation of the subsequent portion of the contraction (4-7, 10, 13). This deactivation has been demonstrated by maneuvers that result in an increase in the velocity of shortening of the muscle...
Effects of caffeine, increased Ca^{2+} concentrations, and tetanus on the time course of activation and isometric force (solid lines) after quick releases in one representative muscle: control and in the presence of 10 mM caffeine (top), at 10 mM caffeine and 7.5 mM Ca^{2+} and in the tetanus (bottom). The presentation is the same as it is in Figure 2. The delayed onset of activation in the presence of caffeine is partially reversed if the Ca^{2+} concentration is raised to 7.5 mM. The dissociation in time of activation and isometric force development in the control is progressively reduced at 10 mM caffeine, at 10 mM caffeine and 7.5 mM Ca^{2+}, and at tetanisation. Deactivation following undamped quick releases compared with slow releases is decreased in the same order. In tetanus, the slopes of activation and isometric force are approximately parallel and deactivation is inhibited.

**FIGURE 6**

Redevelopment of isometric tension after quick releases to preload with subsequent stretch back to the original length at various times after stimulation. The quick length changes totally deactivate contraction in the control muscles at times later than the peak of the twitch (A), whereas in the presence of 10 mM caffeine and 7.5 mM Ca^{2+} (B, C) tension is redeveloped well beyond the time to peak tension.
after a release, when the velocity of shortening has been corrected for series elasticity and measured at the same time and with the same load. Thus, when the rapid motion of the lever after the release is slowed by damping, deactivation is partially prevented. The type of damping and its timing is critical. Furthermore, when the extent of the rapid shortening is reduced by releasing to a greater afterload, the amount of deactivation is also decreased. The latter observation is consonant with the finding that the duration of contraction is prolonged as afterload is increased in isotonically contracting muscles (2, 21, 22).

Following these observations, we worked on the premise that the quick release not only may discharge the stretched SE but also may induce motion and subsequent breakage of contractile crossbridges. For subsequent contraction to occur, these force-generating bridges must be reformed. If Ca²⁺ is removed from the sites that are necessary for activation when the bridges are broken, it would have to be rebound for bridge reformation to occur. We have hypothesized that when the bridge is broken some of the Ca²⁺ involved in activation is released from the contractile sites and subsequently sequestered by the sarcoplasmic reticulum, thus becoming unavailable for reactivation. This removal of Ca²⁺ from the myoplasm would produce the observed deactivation. If this hypothesis is true, factors which increase myoplasmic Ca²⁺ concentration either by inhibiting function of the sarcoplasmic reticulum or increasing the transsarcolemmal influx of Ca²⁺ should reduce the degree of deactivation. This conclusion is supported by the finding that lowering the temperature or adding caffeine, both of which partially inhibit function of the sarcoplasmic reticulum (9, 18-20), reduce deactivation. Caffeine also increases the transsarcolemmal flux of Ca²⁺ and thus raises the myoplasmic Ca²⁺ concentration (9). Tetanization of heart muscle in the presence of caffeine and elevated levels of calcium also eliminates the deactivation following quick releases.

Deactivation due to length changes in contraction (14) is also shown in this study. This deactivation suggests that the Ca²⁺ concentration at contractile sites has already decreased at that time as a result of Ca²⁺ uptake by the sarcoplasmic reticulum. Uptake of Ca²⁺ by the sarcoplasmic reticulum early in contraction has also been shown in skeletal muscle (23). This finding is consonant with the finding that Ca²⁺ used for activation is abruptly released from internal stores at an early time in contraction (24, 25). Since the slow inward current of Ca²⁺ across the sarcolemma presumably contributes relatively little to direct activation (24, 25), the early uptake of Ca²⁺ back into internal stores manifests itself in the observed deactivation.

Consideration of deactivation is important when the relation between force and velocity of shortening obtained by quick releases is used to define contractility or to denote the course of activation (active state) relative to time (4-8, 12-15). The present study suggests that force-velocity curves obtained by quick releases at a constant time in contraction are greatly affected by deactivation and other factors. Thus, in cat papillary muscle, the velocity of shortening that is measured after quick releases to very light loads rises to a peak early along the rising phase of force development (4). The curves obtained by release to heavier loads seem to peak somewhat later in time (4). Moreover, the curves do not fall proportionately with time (Fig. 3). The curve of activation that is determined with light loads falls off rapidly and reaches zero quite early in the time course of contraction; it may transect the other curves obtained with heavy loads. Variable degrees of deactivation greatly affect the course of activation for different loads, and thus the shape of the force-velocity curve changes with time. Indeed, at peak isometric contraction (30°C), the curve becomes incomprehensible (Fig. 3). Thus, as demonstrated in Figure 3, curves derived from quick releases relating force and velocity of shortening at various times during the course of contraction (instantaneous force-velocity curves) are not shifted in a parallel manner due to a time dissociation in capacity to shorten and ability to generate isometric force. Since all measurements were not made immediately after a release, but at a common length to which the CE had shortened 40-50 msec after the peak of the twitch, these findings could be due to the much faster decline in velocity of shortening with light rather than with heavy loads. However, Figure 9 indicates otherwise. The instantaneous velocity of shortening was measured 15 msec after damped quick releases to increasing afterloads at selected times during the contraction (a, b, c, d, e). Since instantaneous force-velocity curves are obtained at a constant time in contraction, corrections of the CE length cannot be made; however, the difference in length of the CE at different loads is negligible (8, 15). The force-velocity relations obtained in the control contraction are shown in Figure 9A. Early after stimulation, the curve had high velocities.
Myocardial deactivation by quick release

Effect of tetanus on instantaneous force-velocity curves. Instantaneous force-velocity relations were obtained from damped quick releases occurring at selected times after stimulation by determining velocity of shortening 15 msec after the release as a function of increasing afterloads. In the control muscle (A) the curves are not shifted in parallel with time after stimulation. In tetanus (B), the curves derived from onset and decline of activation (time to the plateau of activation was 1,680 msec) are shifted in parallel. Curves have been fitted by eye. Lmax = 6.1 mm, cross-sectional area = 1.3 mm², temperature = 23.4°C, resting tension = 0.8 g, and rate = 6/min in the control. Tetanus was obtained by repetitive pulses, 10/sec for 3 seconds, in the presence of 10 mM caffeine and 7.5 mM Ca²⁺. Times indicated are times when the release occurred.

Figure 9

With small loads and fell off rapidly as the load was increased (a). As time progressed, the nature of the curve changed as the velocities measured with light loads decreased and maximum force increased (b, c, d). The relations determined after the peak of isometric contraction were shifted downward at low loads, apparently due to deactivation. The instantaneous force-velocity curves measured at the onset and the decline of tetanus, however, were shifted in parallel (Fig. 9B), since the dissociation in time of capacity to shorten and ability to generate isometric force was eliminated. Moreover, the velocities of shortening at low loads were not depressed during relaxation (Fig. 9B, curve e, 2,000 msec after the last stimulus), suggesting that deactivation was inhibited. This finding does not negate the possibility that, in the absence of caffeine, activation may produce maximum velocity of shortening with light loads quite early in contraction due to the formation of a few bridges, although the capacity to develop maximum force, which requires a large number of bridges, may occur later in time. However, the true time course of velocity of shortening with light loads remains uncertain due to deactivation.

In rabbit papillary muscle, it has been demonstrated that deactivation following quick releases can be inhibited by damping of the lever movement and that instantaneous force-velocity curves are hyperbolic (15). Moreover, such curves are shifted in parallel throughout the contraction (8, 15). It has been argued that the differing results obtained in cat papillary muscle (4, 9, 10) are due to the fact that the lever was not damped (15). However, in a previous study (4) and in the present study, the results are different despite the damping of the lever movement. Clearly, deactivation in rabbit papillary muscle is smaller because the extent of release is not greater than 2 g (15); deactivation is also reduced in cat papillary muscle after small releases as shown in the present study. Furthermore, if the velocity of shortening after damped and underdamped quick releases before the peak of isometric contraction are compared at the same length but not at the same time (Fig. 4A and C, ref. 15), no deactivation can be ascertained. In the later releases (Fig. 4B and D, ref. 15), deactivation is overestimated for the same reason. Deactivation after undamped quick releases compared with deactivation after damped releases would also have been overestimated in the present study if length corrections had not been made. Furthermore, differences between rabbit and cat papillary muscle may be explained by the fact that activation is slower in onset and decline in the rabbit; in that species activation tends to parallel isometric force (26). This finding explains the parallel shift of instantaneous force-velocity curves in the contraction of the rabbit papillary muscle, in which deactivation can also be inhibited by damping of the lever movement, because activation is preserved longer (8, 26).

To measure the course of activation with the quick-release technique measurements of velocity

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of shortening have been made at the same CE length (8). The length to which the CE has shortened at the peak of the isometric twitch has been proposed as a reference length for all measurements at different times in contraction (8). This length represents a point on the active state curve, as originally defined (27), where the CE is neither lengthening nor shortening. Since it has been reported that force and velocity rise and decline in parallel, the velocity of shortening measured in this manner was thought to represent the active state of cardiac muscle (8). However, we did not use this length as the reference because (1) in the cat papillary muscle force and velocity of shortening do not rise in parallel and (2) quick releases made near the peak of the isometric contraction are followed by the greatest recoil of the SE and, hence, the largest inertial oscillations of the lever. These findings complicate accurate assessment of velocity of shortening by extrapolation back through the oscillations. Therefore, we have been unable to measure velocity of shortening earlier than 40-50 msec after an undamped quick release occurring at the peak of the twitch. Thus, the length to which the CE has shortened at that time was used in this study as the reference for all measurements at different times in a contraction. The length corresponded to about 88-90% of Lmax, since the CE had undergone internal shortening of 5-7% and external shortening of as much as 5% when the measurement was made. At these lengths, velocity of shortening may still be independent of length (17) or just starting to decline (16). The conclusions reached in this study would not be altered even if velocity of shortening had been measured at shorter CE lengths, since the hyperbolic shape of the force-velocity relation remains at these lengths (16). Moreover, velocity of shortening would be reduced at the shorter CE lengths in the control contraction, and in the presence of caffeine, and in tetanus (16).

The present study supports the view that deactivation after quick releases is due to uncoupling of crossbridges in contracting heart muscle. This deactivation may be overcome if the Ca²⁺ concentration at these sites remains high enough to permit reformation of these crossbridges. Moreover, consideration of deactivation and the time course of activation are essential for evaluation of instantaneous force-velocity relations obtained by quick-release techniques at a constant time during contraction. It is clear that instantaneous force-velocity curves in cat papillary muscle are influenced by multiple factors (4, 9, 10). Thus, such curves are limited as specific indexes of myocardial contractility (11, 12).

References


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