Active State in Heart Muscle

Its delayed onset and modification by inotropic agents

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ABSTRACT The course of active state in heart muscle has been analyzed using a modified quick release method. The onset of maximum active state was found to be delayed, requiring 110–500 msec from time of stimulation, while the time to peak isometric tension required 250–650 msec. Further, the time from stimulation to peak tension was linearly related to the time required to establish maximum intensity of active state as well as to the duration of maximum active state. The duration of maximum active state was prolonged (90–220 msec), occupying most of the latter half of the rising phase of the isometric contraction. Norepinephrine (10^{-5} M) shortened the latency from electrical stimulus to mechanical response, accelerated the onset of maximum active state, increased its intensity, decreased its duration, and accelerated its rate of decline. These changes were accompanied by an increase in the rate of tension development and the tension developed while the time from stimulation to peak isometric tension was abbreviated. Similar findings were shown for strophanthidin (1 μg/ml) although lesser decrements in the duration of maximum active state and time to peak tension were found than with norepinephrine for similar increments in the maximum intensity of active state.

The intensity of active state of the contractile elements of muscle may be defined as a mechanical measure of those chemical processes at contractile sites which generate force and shortening (1); it can be characterized mechanically by the relations between force and velocity of shortening at any instantaneous muscle length both at the start and during the course of contraction (2, 3). Thus, a complete mechanical description of the active state requires the measurement of three interrelated variables, *force*, *velocity*, and *muscle length*, as functions of *time* (4).

The fact that the intensity of active state in heart muscle may be altered is already apparent from the finding that the force-velocity relation can be shifted by changing frequency of contraction (5), or the addition of calcium or norepinephrine (6, 7). The first direct studies of the active state in heart muscle were carried out by Abbott and Mommaerts (5), who found that the rate of decline of active state could be accelerated by increasing contraction

frequency. However, the methods employed did not permit evaluation of the onset of active state or a clear delineation of changes in its intensity or duration. Other studies (8, 9) with similar methods have indicated that the duration of active state is unaltered by changing initial muscle length but is abbreviated by norepinephrine (9). The demonstration that quick stretches have not yielded maximum isometric force early in the course of contraction in heart muscle (5, 10), in contrast to skeletal muscle (11), has raised the question whether there is a delay in the onset of maximum active state in heart muscle (10). Since quick stretch of the muscle permits examination of only those aspects of active state reflecting force, and since the quick stretch itself may also alter the active state, at least for insect muscle (12), this suggestion has remained unsettled. However, preliminary observations (4, 13) have already lent additional credence to the view that the onset of active state in heart muscle is delayed.

The present study was undertaken to determine the course of active state in heart muscle and the effects of various inotropic interventions, such as norepinephrine and strophanthidin, on its onset, maximum intensity, duration, and decay. Accordingly, active state has been studied using a modified quick release method (2). Velocity of shortening following quick release has been measured at multiple points in time following stimulation while holding afterload (or force) and muscle length constant. Under such conditions, the instantaneous velocity of shortening after quick release serves as a relative measure of the intensity of active state and the course of active state has been explored.

METHODS

Papillary muscles removed from the right ventricles of cats anesthetized with sodium pentobarbital (25 mg/kg) were used in this study. Details of this preparation have been described previously (6). A diagram of the apparatus employed is shown in Fig. 1. The muscle was placed in a myograph containing Krebs' solution (6) which was aerated with 95% oxygen and 5% carbon dioxide. The lower, nontendinous end of the papillary muscle was held firmly in a spring-loaded Lucite clip that formed the end of a steel tube which passed through the bath bottom and attached directly to a force transducer (Statham Model G 1-4-350). The upper, tendinous end of the muscle was tied directly to a wire extension from an isotonic lever. The isotonic lever used to measure displacement in these studies has been described elsewhere (14). The lever, fashioned from magnesium, was 20 mm in length with a 20:1 reduction ratio and had an equivalent mass of 45 mg. In order to reduce inertia further, weights were attached across the fulcrum of the lever through a long segment of distensible rubber. Displacement of the lever was measured with a photodiode system which was linear over 2 mm. The velocity of displacement was differentiated electronically using an RC circuit (1.0 msec time constant) and in some instances a Philbrick operational amplifier.

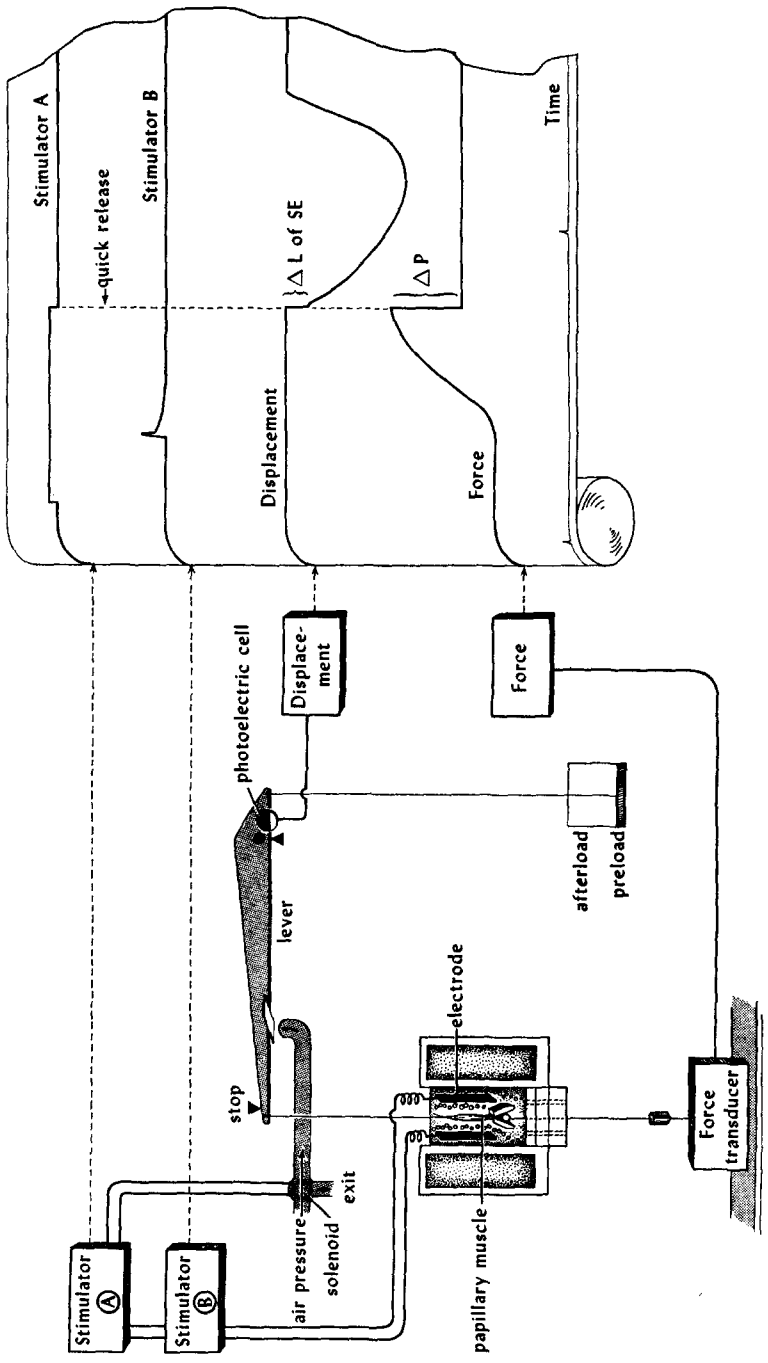

FIGURE 1. Diagram of apparatus for studying afterloaded isotonic contractions with quick releases. ΔL = the rapid displacement immediately following quick release; ΔP = the fall in force following quick release, following which force equals the afterload (P).

In order to determine the intensity of active state, a quick release system was employed after the manner of Jewell and Wilkie (2). A jet of air controlled by a solenoid valve was used to keep the lever fixed until a desired time after stimulation of the muscle (Fig. 1). The use of an air jet rather than a mechanical release system afforded the advantages of minimal noise and the ability to recycle the process rapidly so as to maintain a necessary steady frequency of contraction. Prior to stimulation of the muscle the jet was directed against the lever by stimulator A,[1] which controlled the solenoid (Fig. 1). After an appropriate delay, stimulator A triggered stimulator B, which delivered a mass-stimulating pulse 1.5 times threshold to the muscle through platinum plates arranged along the lateral aspects of the muscle (Fig. 1, right). A stimulation of

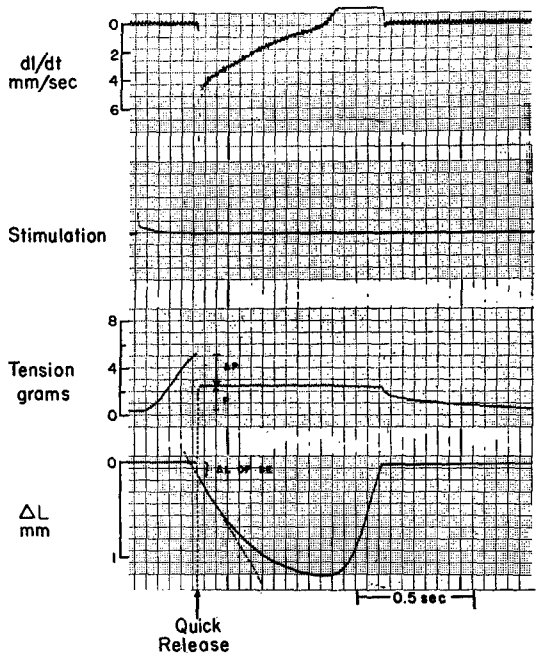

FIGURE 2. An afterloaded isotonic contraction with quick release occurring 250 msec after stimulation. From above down are recorded velocity of isotonic shortening (dl/dt), stimulation artifact, tension, and displacement (ΔL). The point of quick release is noted. P and ΔP as defined in Fig. 1.

12/min was employed throughout. Lever displacement, its differential (dl/dt), force, and the stimulation artifact were recorded simultaneously on a multichannel oscillograph and simultaneously on a Tektronix dual beam oscilloscope (Model 502), where they could be photographed as desired.

The intensity of active state was determined from the isotonic contraction by using a constant preload and afterload, and then determining the velocity of isotonic shortening after quick release, immediately following the rapid initial transient of series elastic shortening (2). In Fig. 2, the course of a single contraction with quick release is shown. Following quick release, 3–4 msec were required for isometric force to fall to a steady state. By varying the time after stimulation of the muscle at which quick release occurred and plotting instantaneous velocity of shortening as a function of this release

[1] Laboratory Stimulator Model *104A*, American Electronics Laboratories, Colmar, Pa.

time the course of active state intensity of the muscle was obtained. The peak instantaneous velocity of shortening following quick release represents the maximum intensity of active state. The duration of maximum intensity of active state was taken as the time during which 95% of this peak velocity of shortening was maintained. With these methods, the effects of changing afterload and of the addition of norepinephrine and strophanthidin on the course of the active state were evaluated. A temperature of 27°C was maintained and a preload of 0.5 g was employed routinely.

RESULTS

The Onset and Course of Active State

The course of active state has been explored in 182 experiments on 15 cat papillary muscles. A typical experiment is illustrated in Fig. 3. A preload of 0.5 g and an afterload of 2.0 g were used. Repetitive quick releases were performed at 10 msec intervals. Both isometric tension and instantaneous velocity of shortening following quick release are shown as functions of time after stimulation. Each point represents a quick release as shown in Fig. 2, and the change in instantaneous velocity of shortening after quick release indicates the intensity of active state as a function of time after stimulation. In the process of muscle shortening, a steady-state velocity of shortening was not maintained as seen in Fig. 2 and the peak velocity after release was measured.

In 15 papillary muscles, latency from stimulation to the first evidence of force development averaged 35 ± 6 (SE) msec while the time to peak isometric tension averaged 520 ± 27 msec. The onset of maximum active state was always delayed, the time to 95% of the maximum intensity of active state with moderate afterloads (1.5–3.0 g) averaging 282 ± 22 msec and the time to maximum intensity of active state averaging 399 ± 24 msec. The duration of maximum intensity of active state, as measured by the time during which at least 95% of the maximum instantaneous velocity of shortening was maintained, was found to be 174 ± 14 msec. The active state declined below 95% of maximum 456 ± 18 msec after stimulation. Thus the decline of maximum active state generally occurred just prior to maximum isometric force development.

Both the time necessary to attain the maximum intensity of active state and the duration of maximum active state were found to vary in a linear manner with the time from stimulation to maximum isometric force (Fig. 4). The data in Fig. 4 represent all experiments from 15 muscles performed with an afterload of 2.0 g and preload of 0.5 g, including those following such inotropic interventions as the addition of norepinephrine or strophanthidin. The deviation between the line for the time to 95% maximum active state and the line for the decline to 95% of the maximum active state represents the approximate duration of maximum active state. Thus the duration of maximum active state is also a linear function of the time to peak tension.

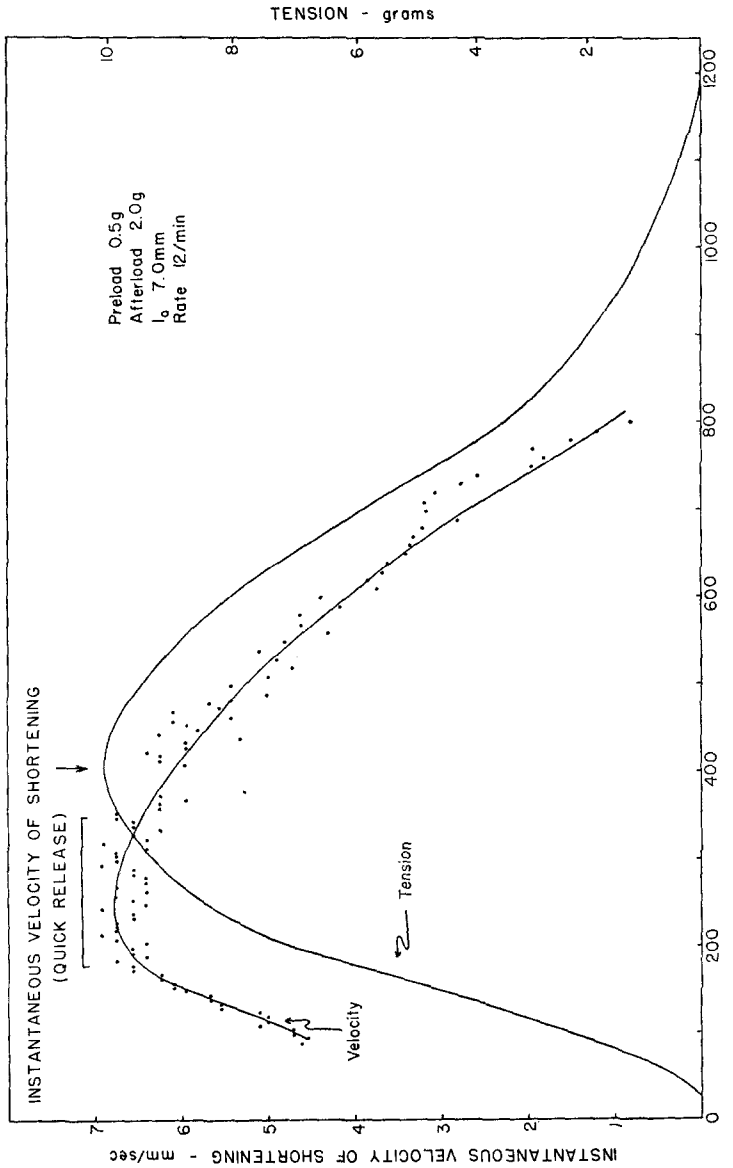

FIGURE 3. The course of active state and isometric tension relative to time after stimulation. The latency from stimulation to the first evidence of force development was 35 msec and maximum force development required 400 msec. Active state was determined at each point from the instantaneous velocity of shortening following quick release. 95% of the maximum intensity of active state is attained only after 180 msec and maintained for 170 msec (bracket). Active state falls below 95% of maximum after 350 msec. Muscle cross-sectional area 0.92 mm.^{2} l_o = muscle length.

Effects of Changing Load on Active State

In 16 experiments on six muscles, the effects of changing afterload on the course of active state were explored as in Fig. 5. With very light loads, namely

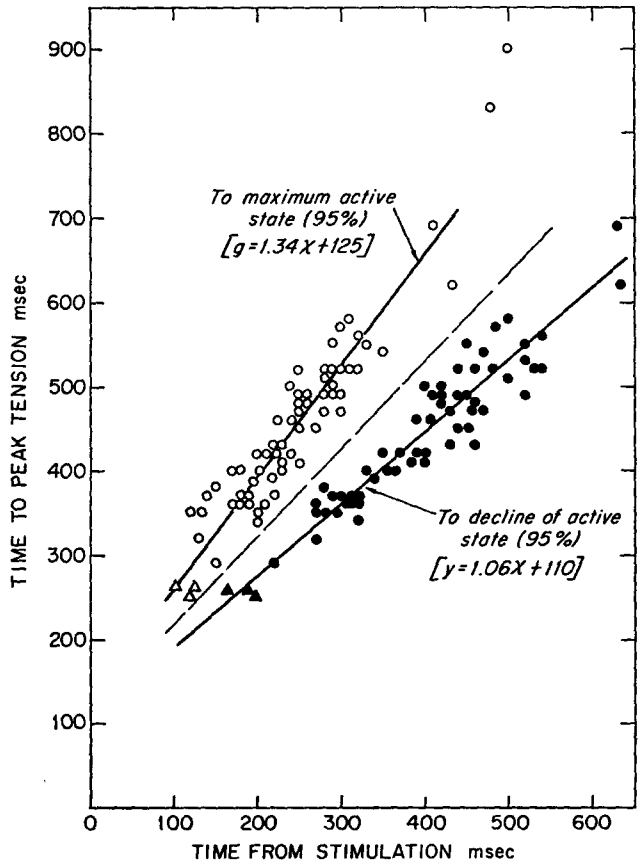

FIGURE 4. The relation of time to peak isometric tension to the time to reach 95% of the maximum intensity of active state (○), the time to maximum intensity of active state (– – –), and the time to decline to 95% of maximum active state (●). The triangles represent three experiments performed at 37°C. The longitudinal distance between the time to development of 95% maximum intensity of active state and the decline to 95% of maximum active state represents the duration of maximum active state.

with the preload alone and afterloads of 1.0 g or less, maximum intensity of active state was reached slightly earlier and was maintained for a somewhat briefer period of time than was observed with heavier loads. Once a moderate load was established, i.e. an afterload greater than 1.0 g, the onset and duration of maximum intensity of active state were only slightly affected by further increasing the afterload. From such experiments, the relation between force (load) and velocity was shown to vary with time throughout the

contraction (Fig. 6). However, an inverse relation between force and velocity of shortening pertained throughout the development of tension, and once maximum active state was attained, a relatively stable force-velocity relation

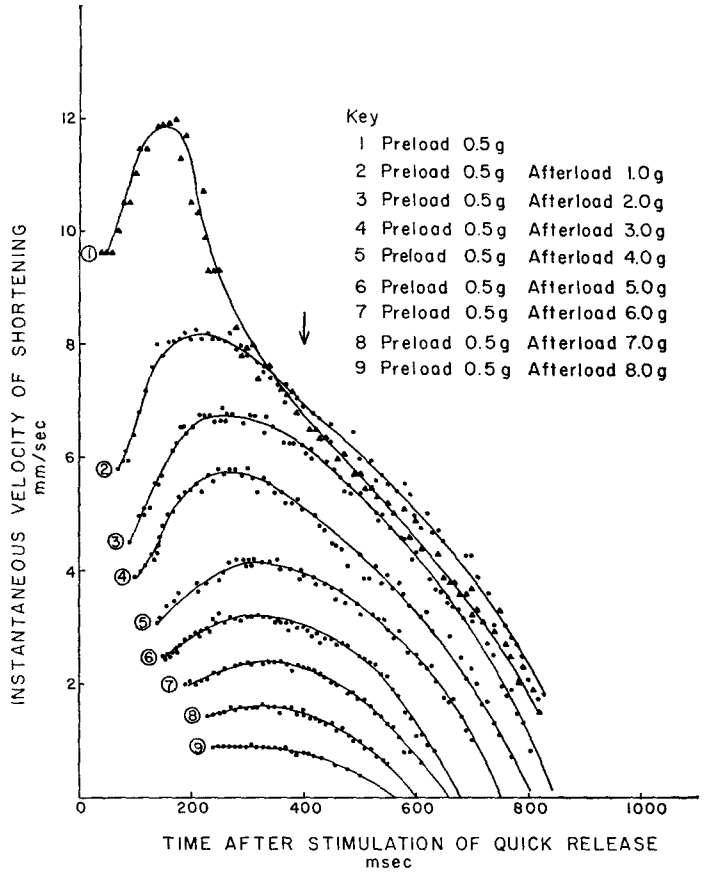

FIGURE 5. The effects of increasing afterload on the course of active state. The instantaneous velocity of shortening following quick release is shown as a function of time after stimulation. Each point represents an experimental measurement. The vertical arrow (↓) represents the time required to reach maximum isometric tension. Muscle length, 7.0 mm; cross-sectional area, 0.9 mm^2; P_0 (maximum isometric force), 9.6 g/mm^2.

was found for approximately 200 msec, that is during the period that the maximum intensity of active state was maintained. In six experiments on two muscles, quick release velocity studied with the preload alone tended to fall sooner than with heavier loads. However, in 10 experiments on four muscles, quick release velocity was greater with light loads than with heavier loads throughout the contraction.

Effect of Norepinephrine and Strophanthidin on the Course of Active State

The effects of norepinephrine and strophanthidin on the course of active state were studied in nine and four preparations respectively. Typical results from each intervention are shown in Figs. 7 and 8, with the course of active state shown above and the concurrent isometric contraction shown below, both as functions of time after stimulation. The addition of nor-

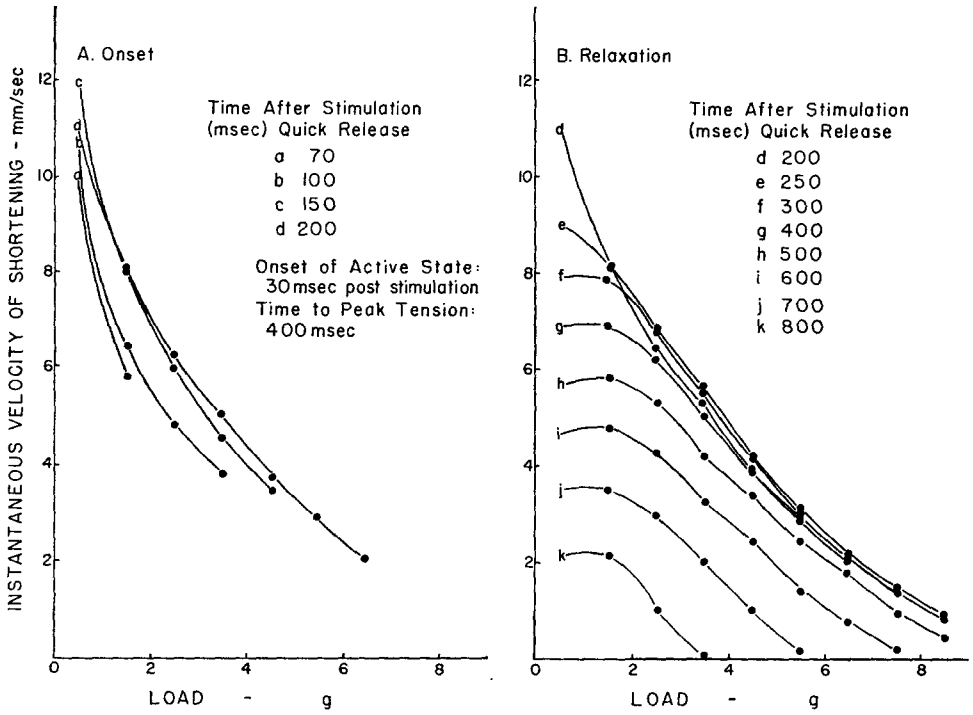

FIGURE 6. Instantaneous force-velocity relations obtained from quick releases by determining velocity of shortening as a function of increasing afterloads at selected times after stimulation. From experiment shown in fig. 5, panel A represents the force-velocity curves during the onset of active state while panel B represents the phase of relaxation.

epinephrine (10^{-6} M) accelerated the onset of maximum active state, increased its maximum intensity, shortened the duration of maximum active state, and hastened its decline (Figs. 7 and 9 A). Following the addition of norepinephrine in all nine experiments, latency between stimulation and mechanical activity decreased from 39 to 22 msec [mean change, -17 ± 2 (SE) msec] and time to peak isometric tension decreased from 504 to 409 msec (mean change, -95 ± 4 msec) while peak isometric force increased $27 \pm 2\%$. The time to reach maximum active state decreased from an average of 386 to 288 msec (mean change, -98 ± 5 msec), and time to attain 95%

of maximum active state decreased from 312 to 222 msec (mean change, −90 ± 6 msec), while the maximum intensity of active state was increased by an average of 48 ± 6%; the duration of maximum active state was shortened

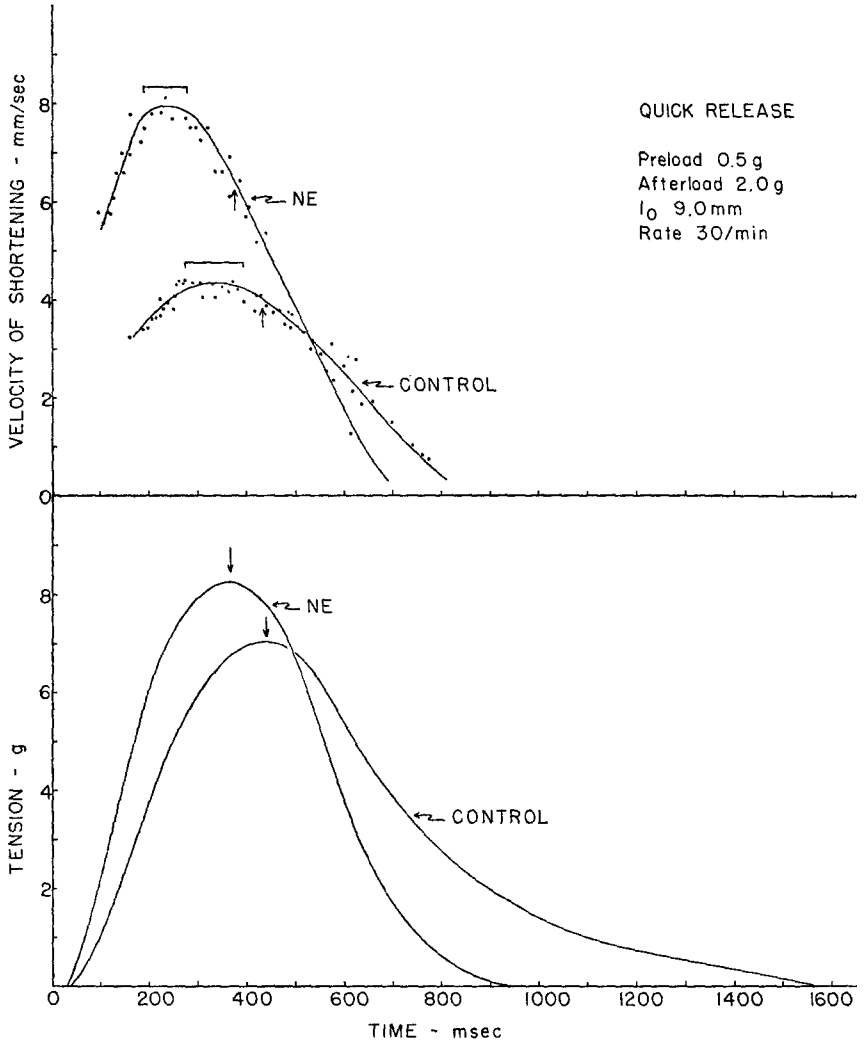

FIGURE 7. The effects of norepinephrine (NE) (10^{-6} M) on the course of active state (above) and isometric tension (below). The brackets represent 95% of maximum intensity of active state while the vertical arrows represent the time to maximum isometric tension. l_o = muscle length.

from 179 to 134 msec (mean change, −45 ± 3 msec), and the rate of decline of active state was increased by 88 ± 11%. Thus the more rapid onset, increase in maximum intensity, and abbreviation of the duration of maximum active state following norepinephrine were accompanied by an increase in

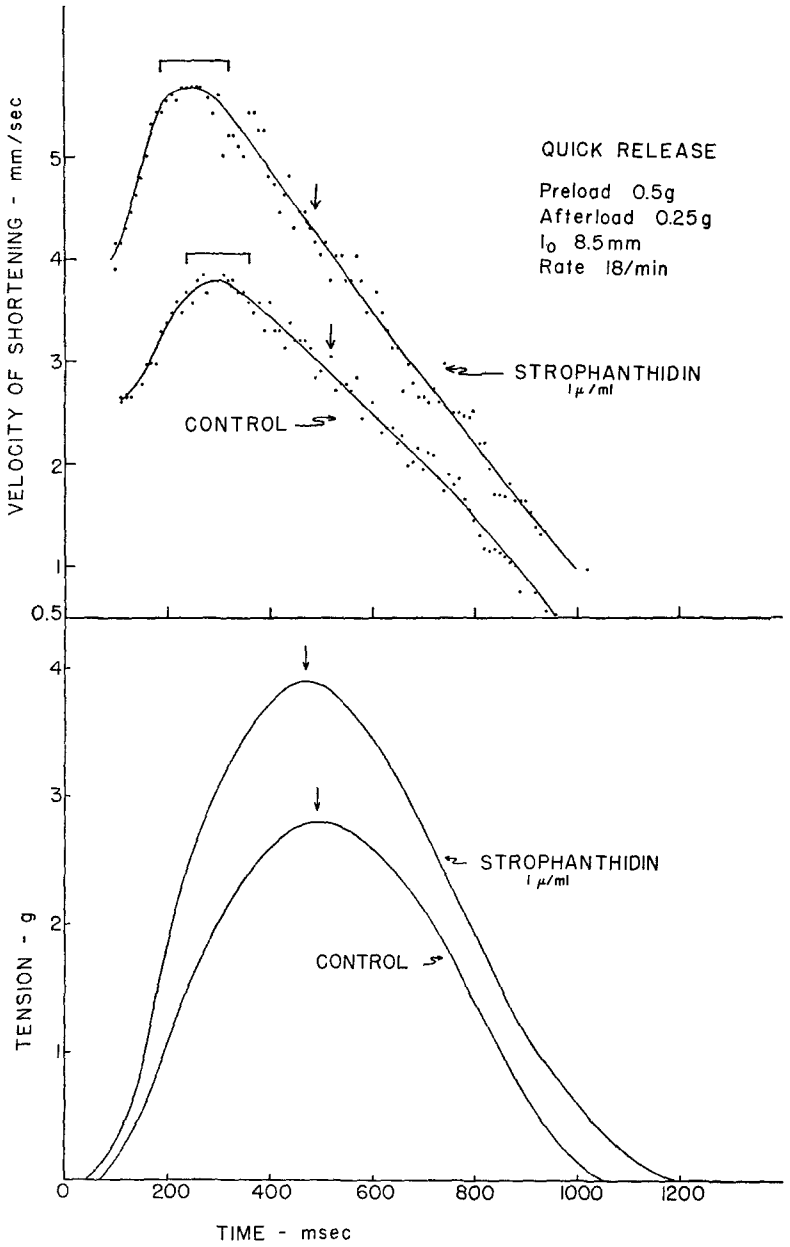

FIGURE 8. The effects of strophanthidin (1 μg/ml) on the course of active state (above) and isometric tension (below). The vertical arrows and brackets have the same meaning as in Fig. 7.

the maximum rate of tension development (68 ± 8%) and an increase in developed tension (27 ± 2%) along with a decrease in the time from stimulation to maximum isometric tension (14 ± 2%).

The addition of strophanthidin (1.0 μg/ml) to the bath (Figs. 8 and 9 B) also resulted in an augmentation in the maximum force of isometric contraction (24 ± 4%) as well as an increase in the maximum intensity of the active state (30 ± 6%). In contrast to norepinephrine, this was accomplished with a smaller decrease in the time from stimulation to maximum isometric

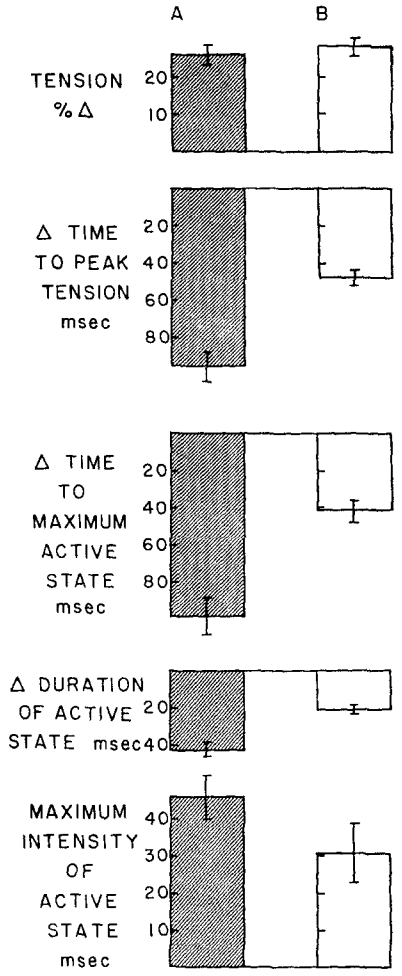

FIGURE 9. The effects of norepinephrine (A) and strophanthidin (B) on various aspects of isometric tension development and the course of active state. The vertical brackets represent 1 SEM.

force from an average of 482 to 434 msec (mean change, −48 ± 2). Compared with norepinephrine, a smaller decrease in the time required for the onset of maximum active state was noted following strophanthidin, the time to maximum active state decreasing from an average of 355 msec to 313 msec (mean change, −42 ± 3 msec), and the time to 95% of maximum active state decreasing from an average of 268 msec to 223 msec (mean change, −43 ± 4 msec). The duration of maximum active state was also

decreased slightly from an average of 165 msec to 144 msec (mean change, -21 ± 1 msec).

DISCUSSION

The present study serves to support the view that the development of the maximum intensity of active state in heart muscle is a slow process (4, 10, 13). Thus time becomes an important factor in determining the force–velocity–instantaneous muscle length relation of myocardium (3), which has been useful in defining the contractile state of the heart. This delay in the onset of active state was also implicit in the quick stretch studies of Abbott and Mommaerts (5) as demonstrated by Brady (10).

The present study has also demonstrated that this delay in the onset of maximum active state is not constant but may be abbreviated by inotropic interventions such as norepinephrine. Norepinephrine not only accelerates the onset of active state, but increases its maximum intensity while shortening its duration. The rate of decline of the active state is also augmented by norepinephrine. The acceleration of the onset and abbreviation of the duration of active state are reflected in a decrease in the time to peak isometric tension while increments in the maximum intensity of active state accompany an increase in maximum force development.

Like norepinephrine, strophanthidin increases the maximum intensity of active state and augments the force of contraction in heart muscle, but, in contrast to norepinephrine, strophanthidin produces little change in either the time required to develop maximum active state or the duration of active state. This is further reflected in the observation that the time from stimulation to maximum isometric tension is only slightly abbreviated following strophanthidin. Thus, although a shortened duration of the active state, and hence shortened duration of contraction, generally accompanies an increase in the intensity of the active state following an inotropic intervention, augmentation of the maximum active state need not be accompanied by such an abbreviation of the active state.

The finding that the course of isometric tension directly reflects the course of active state is useful in assessing the contractile state of heart muscle. The time to reach peak isometric tension is directly proportional to the time required to develop maximum active state and the duration of maximum active state. Further, an increase in the rate of isometric force development at a given muscle length reflects an increase in the maximum intensity of active state. Thus, an analysis of the rate of force development and the time required to develop maximum isometric tension provides an indication of the intensity and duration of active state.

In skeletal muscle, the force of contraction in the twitch may be augmented by various interventions (15–18). However, unlike cardiac muscle, where

the intensity of the active state is augmented while the duration of active state is unchanged or abbreviated, in skeletal muscle the duration of active state may be prolonged without a change in the maximum intensity (16–18). In contrast, in heart muscle, inotropic interventions such as norepinephrine shorten the duration of contraction while increasing velocity of contraction, again resulting in increased force of contraction. These findings are consonant with the observations that the force-velocity relations of skeletal muscle are unalterable under physiological conditions (1) while the force-velocity relation in heart muscle is readily altered with changes in maximum velocity of shortening (V_{max}) by such interventions as alterations of heart rate (5, 6), calcium (6, 7), or norepinephrine (6, 7).

Certain limitations to the present methodology should also be noted, as discussed by Brady (13). Although over-all muscle length is held constant prior to quick release, some contractile element shortening has already ensued. Thus the quick release method does not reveal the velocity of shortening at a constant contractile element length. However, such shortening of the contractile elements would tend to decrease the measured velocities progressively while force is increasing, and thus the delay in the onset of active state would actually be greater than that which has been demonstrated. From the same reasoning, the duration of maximum active state could conceivably be somewhat longer than observed. However, this would not alter the general conclusions.

An explanation for the delay in the onset of active state and its modification by inotropic interventions in heart muscle remains speculative. Considering the rapidity with which active state was developed in skeletal muscle, A. V. Hill suggested that diffusion of an activating substance from a superficial site along a skeletal fiber 100 μ in diameter could not occur rapidly enough to account for activation (19). However, present concepts (16) would suggest in skeletal muscle that the sarcoplasmic reticulum (20–22) releases calcium adjacent to each sarcomere which diffuses in and induces contraction. In heart muscle, the sarcoplasmic reticulum is much less profuse than in skeletal muscle (23) and whether it subserves the function of activation in the same manner remains uncertain. However, the cardiac cell or fiber has a diameter of only 5–10 μ, and, it is conceivable that activation follows release of an activating substance directly from a relatively superficial site. This might explain the delayed onset of maximum active state in heart muscle.

If activation were to occur by a diffusion process, it would be expected that maximum velocity with small loads would occur sooner than maximum force; such is observed. Inotropic interventions such as norepinephrine may alter this diffusion process, perhaps by making active membranes more permeable, leading to the more rapid diffusion of an activator to contractile sites. The manner in which the more rapid onset of active state following an

inotropic intervention is coupled to a shortened duration of maximum active state and an accelerated rate of decline is unexplained. Merely increasing the amount of activator reaching the contractile sites might explain an increase in the rate of interactions at these sites, and hence an increase in the velocity of contraction, but would not shorten the duration. The acceleration of relaxation following norepinephrine may result from another separate action of the intervention which is as yet undefined.

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