Effects of Altered Loading on Contractile Events in Isolated Cat Papillary Muscle

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ABSTRACT

When the mode of contraction of the cat papillary muscle is changed abruptly from isotonic to isometric, the tension of the first isometric contraction is as much as 22% greater and lasts substantially longer than the subsequent stable isometric contractions attained after a few beats. This previously undescribed phenomenon is largely independent of preload or inotropic influences, but is greatly diminished at lower temperatures. Force-velocity curves equivalent to the first isometric contraction revealed a maximum velocity of shortening 9.5 ± 2.0% greater than that of the stable isometric contraction. Thus apparent changes in muscle contractility can occur whenever there are sudden substantial changes in tension development. This effect may be due to transitory changes in free intracellular calcium or, alternatively, to the presence of a viscous element in close association with the contractile element.

ADDITIONAL KEY WORDS

viscous element contractility
isotonic isometric paired stimulation
homeometric autoregulation V_max

On the basis of the studies in skeletal muscle of A. V. Hill and others (1, 2), the mechanical properties of heart muscle have been analyzed (3, 4). The relationship between the velocity of shortening and the load (force-velocity relation) has been particularly useful. Thus the tension developed and the maximum velocity of shortening (V_max) at zero load are both increased by inotropic influences such as catecholamines, calcium, or digitalis (5, 6). Changes in initial muscle length (preload) also produce changes in tension development, but do not alter V_max. Thus, although both an increase in initial muscle length and an inotropic influence may increase tension development, only the latter will change V_max, which can therefore be used as an index of contractility (4).

In most studies of isolated heart muscle, measurements have been made under stable loading conditions, and it has been assumed that in the absence of an inotropic influence contractility remains constant, independent of preload and afterload. However, in the present study, a sudden change in the mode of contraction from isotonic to isometric revealed in the first isometric contraction that there had been a heightened contractile state during the isotonic contraction; this heightened contractile state then disappeared with time. This phenomenon may be due to a transient increase in available calcium, or alternatively, to a viscous element in close association with the contractile elements.

Methods

Forty right ventricular cat papillary muscles with cross-sectional areas of 1.4 mm² (7) were studied in vitro. Cats were anesthetized with sodium pentobarbital, 50 mg/kg, ip. Papillary muscles were rapidly removed from the right ventricle and placed in a muscle bath filled with Krebs bicarbonate solution at a constant temperature of 29°C and aerated with 95% O₂-5% CO₂.
The nontendinous end of the muscle was placed in a spring-loaded lucite clip which formed a rigid extension of a tension transducer. The muscle-tendon junction was placed in a light metal clip (mass 70 mg), the extension of which was attached directly to the tip of an isotonic lever system (equivalent mass, 70 mg). Both tension and length were differentiated electronically to provide the rate of change of tension development (dP/dt) and velocity of shortening (dl/dt). The muscle was stimulated at 12 contractions/min by mass electrodes, using pulses of 5 msec and voltages 10% above threshold. Measurements were recorded on a multichannel oscillograph (Hewlett-Packard 7858) at a paper speed of 100 mm/sec, while selected tension traces were also displayed on a memory oscilloscope and photographed for direct comparison. Quick release studies were performed with an air jet apparatus which held the lever against a stop to prevent shortening (8). During such an isometric contraction, the air jet was diverted from the lever at any predetermined time interval, which allowed the muscle to shorten.

The mode of contraction was abruptly changed from isotonic to isometric by leaving the air jet (which holds the lever fixed) turned on throughout the course of contraction.

The decline in tension development following a sudden change from isotonic to isometric contractions was studied in 40 muscles at different preloads, in 25 muscles with the calcium concentration in the bath increased from 2.5 to 7.5 mM, in 13 muscles after the addition of 10⁻⁵ M isoproterenol to the bath, and in 33 muscles at bath temperatures of 24°, 29°, and 34°C. In most studies, tension was also recorded at a higher gain to evaluate changes in diastolic compliance.

The top panel shows tension and rate of tension development (dP/dt) for a series of isometric contractions immediately following a series of isotonic contractions. Fast paper speed is 100 mm/sec; slow paper speed in the middle is .5 mm/sec. The bottom panel shows length change and velocity of shortening (dl/dt) for a series of isotonic contractions without afterload immediately following the isometric contractions of the top panel.

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The theoretical $V_{\text{max}}$ at zero load was calculated from each force-velocity relation by assuming that the first half of the curve from zero tension to one-half of isometric tension was hyperbolic. The equation for the hyperbola was determined from three simultaneous equations utilizing the velocity of shortening at three different loads (preload only, afterload equal to one-fourth isometric tension and one-half isometric tension). The force-velocity curve was then extrapolated to zero tension to obtain $V_{\text{max}}$. Statistical comparisons were done with Student's nonpaired and paired $t$-tests.

Results

A. Isotonic to Isometric Contractions.—The basic phenomena to be described in this paper are illustrated in Figure 1. In the top panel, tension and rate of tension development $(dP/dt)$ are shown for the first and subsequent isometric contractions after a series of isotonic contractions without afterload. Note that the first isometric contraction following isotonic contractions develops considerably more tension than the subsequent contractions, which finally stabilize at a lower level. Similarly, maximum $dP/dt$ and the duration of contraction were always greater for the first isometric contraction. In 30 muscles, the developed tension of the first isometric contraction averaged 8.2 g/mm² and decreased by 1.5 ± 0.1 g/mm² in the stable isometric contraction. The percent change in $dP/dt$ closely paralleled the change in tension development. Time to peak tension was always slightly greater for the first isometric contraction than for the stable isometric contraction. The time from the onset to peak tension in 30 muscles averaged 391 msec for the first isometric beat and 367 msec for the stable isometric beat ($P < .001$), a 6.5% change. The average time for tension to decline to one-half its peak value during relaxation was 427 msec in the first isometric beat and 355 msec in the stable beat, a difference of 20 ± 3%. In the bottom panel of Figure 1, which shows shortening (length) and velocity of shortening $(dl/dt)$, the first and subsequent contractions immediately following the change from an isometric to an isotonic mode of contraction are illustrated. Note that in the first isotonic contraction following the isometric beat, the $dl/dt$ and the distance the muscle shortened were less than the subsequent isotonic beats. These phenomena were reproducible and reversible and were uniformly observed in all 40 muscles. In no instance was this effect absent or an opposite effect observed.

B. Changes in Preload.—The magnitude of the fall in tension between the first and stable isometric contractions at different preloads is illustrated in Figure 2. The top panel shows the average changes at different preloads. As preload was increased, the tension developed on both the first and stable isometric beats was increased (Frank-Starling effect), while the absolute difference between them was not significantly changed. The bottom panel plots the difference in tension as a percent of the stable isometric beat. A change in tension of $22 \pm 2\%$ at the lower preloads fell to $13 \pm 2\%$ at

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**Figure 2**

Upper panel: Average tension developed by the first and stable isometric contractions at different preloads, with the difference shown as the mean ± SEM. Lower panel: Percent increase in tension of the first beat as compared to the stable beat, at different preloads. The number in the bar is the number of muscles in each group.
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higher preloads. These differences reflect the greater absolute value of the tension developed at the higher preloads.

The tension developed on the first isometric contraction at a low preload can be equal to or even greater than the tension developed by stable contractions at the top of the length-active tension curve. In 10 muscles, length-tension curves were obtained for both first isometric and stable isometric contractions. An example is shown in Figure 3. When resting tension was approximately 30% (0.3g) (D) of the resting tension at the top of the length-tension curve (E), the first isometric contraction (A) developed the same tension as did the stable isometric contraction at the top of the length-tension curve (B). Note also that the difference between the first and stable contractions diminished over the top of the curve. With resting tensions greater than 30% of E, the first isometric contraction developed more tension than stable contractions at the top of their length-tension curve. Previous descriptions of the maximum force development at the top of the length-tension curve (7) have always referred to stable isometric contractions, which underestimate the first isometric contraction by an average of 13% (Fig. 2). Further, increasing muscle length alone cannot correct this deficit in stable tension development.

C. Changing Frequency of Stimulation.— Figure 4 shows the change in tension between the first and stable isometric contractions as the frequency of stimulation was increased from 6 to 12 to 24/min. As frequency of stimulation was increased, tension rose in both contractions, while the absolute difference and the percent difference were also significantly augmented (P < .01 when comparing 6/min with 24/min).

The time required for isometric tension to stabilize at a lower level is shown in Figure 5.
The fall in tension as a function of time was roughly exponential for the first few contractions. Further, at the higher frequency, stable tension was attained more rapidly, although more beats were needed for stabilization.

D. Changing Temperature.—Figure 6 shows the difference in developed tension between the first and stable isometric contractions as temperature was progressively increased from 24°C to 29°C to 34°C. Both the absolute and the percent changes at 24°C were substantially less than those at higher temperatures (P < 0.01), while the decline in tension occurred more slowly.

E. Calcium, Isoproterenol, and Paired Stimulation.—In Figure 7 the differences in developed tension between the first and stable isometric contractions are shown under control conditions and during three different potentiated states after (1) the calcium concentration in the bath was increased from 2.5 to 7.5 mM, (2) the addition of isoproterenol (10^{-3} M), and (3) potentiation with paired stimulation (sustained postextrasystolic potentiation). Even with maximum potentiation of the muscle, the absolute change in tension development between the first and the stable isometric contractions was only slightly diminished (top panel). However, the percent change (bottom panel) was somewhat diminished, largely because of the elevated baseline of developed tension produced by these three methods of potentiation.

F. Quick Release Contractions.—With the use of an air jet (8), isometric contraction could be maintained for any period of time. When the air jet was turned off (quick release), the lever was free to move and tension fell abruptly to match the load on the lever. In Figure 8 are superimposed the first and stable isometric contractions produced by maintaining the air jet against the lever throughout the course of contraction. The average tension developed and percent difference between the first and stable isometric contractions at 24°C, 29°C, and 34°C.
substantial reduction in both the tension developed and the duration of contraction between the two are evident. Following another series of isotonic contractions, the first isometric contraction and every subsequent contraction were subjected to quick release. The resulting first and stable quick release contractions, with the time of quick release occurring 300 msec after the onset of tension development, are also shown in Figure 8. Note that there is no change in the course of tension development between the first isometric and both quick release contractions.

In 22 muscles, the time of quick release was increased sequentially by 50-msec increments to obtain first and stable quick release beats throughout the course of contraction. When the time of quick release was less than about two-thirds of the time to peak tension, the first and stable quick release beats were identical. Rarely, the tension developed by the stable quick release contraction was slightly more than that of the first quick release contraction. When the time of quick release reached 69 ± 2% (22 muscles) of the time to peak tension, the stable quick release beat always developed less tension than the first quick release beat. Thus the manifestation of this phenomenon is dependent in a complex manner on the time that tension is maintained.

G. Effect on Force-Velocity Relations.—In isolated muscle preparations, force-velocity relations generally have been obtained by increasing the afterload sequentially and measuring the maximum velocity of shortening at each afterload (4). With the above-mentioned phenomena in mind, velocity of shortening at any load could be altered, depending on the conditions of loading prior to and at the time of measurement. For example, in Figure 9, the first-beat force-
The standard force-velocity curve was determined by sequentially adding afterload and measuring the maximum velocity of shortening. The first and stable beat force-velocity curves are equivalent to the first and stable isometric beats (see text).

The points on this curve were obtained by having the muscle stabilize isometrically first, reducing the load to the desired level, and then measuring the velocity of shortening of the first afterloaded contraction. The force-velocity relations obtained from the isotonic or isometric baseline form two different but almost parallel curves. The usual method of obtaining a force-velocity relation, whereby the afterload is increased sequentially, is actually a composite of these two curves, and is similar to the isotonic curve at low loads and the stable isometric curve at high loads (Fig. 9, broken line). These changes in the force-velocity relations were present in all 40 muscles studied. The calculated $V_{\text{max}}$ at zero load was different for the first beat and stable beat curves ($9.5 \pm 2.0\%$ in six muscles).

H. Series Elastic Element.—In 10 muscles, experiments were performed to determine whether the series elastic element was affected during the transition from the first to the stable isometric contractions. A representative experiment is seen in Figure 10, where the load extension curve of the series elastic element was determined by quick release (8). Several endpoints were obtained for the quick release curves of both the first beat and stable beat. As shown in Figure 10, all points fell on the same curve, indicating that the stress-strain relations of the series elastic element were unaffected.

I. Equipment Compliance.—To eliminate stress relaxation associated with equipment connections and to reduce extraneous compliances, a metal clip was used to attach the
Representative series elastic element extension curve determined by quick release methods. The first and stable contractions fall on the same curve.

Figure 11 illustrates the differences in compliance and viscosity between connections made with 4-0 silk (string) and the special clip. The left-hand side of the figure illustrates the total equipment compliance, when either the 4-0 silk or the clip was used. The compliance observed with string was reduced by approximately 40% with the use of the clip.

The stress relaxation of these connections was examined by imposing various square-wave changes in length on the lever system (right-hand side of Fig. 11). With the thread connections, tension rose abruptly, then fell to a lower level (stress relaxation) due to the viscous properties of the thread and knots. However, with the clip there was a square-wave change in tension similar to the square-wave change in length. Thus, the use of the clip not only reduced equipment compliance significantly but essentially eliminated viscous properties of the connections. Any viscous changes observed during the study could be

FIGURE 10

Preload = .6 g
Muscle Length = 6.2 mm

FIGURE 11

Equipment compliance, using either 4-0 silk (string) or a special metal clip as connections between the muscle and the myograph. Right hand panels show the effect of a change of square-wave length on the string and clip connections. Note the stress relaxation in the tension trace of the string panel.
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wholly ascribed to the muscle and not to the connections with the myograph.

Discussion

The present study has demonstrated that sudden substantial changes in loading (isotonic to isometric) reveal a heightened contractile state of the isotonic mode of contraction. This was manifested in the first isometric contraction by a greater developed tension and rate of tension development than the subsequent isometric contractions. This conclusion assumes that the contractile state of the previous isotonic contraction is the same as the first isometric contraction. Furthermore, the decay in contractile state during subsequent isometric contractions is likewise revealed in the first isotonic contraction by a decrease in the velocity of shortening and the distance shortened (Fig. 1).

Three basic phenomena have been described which occur with time in relation to sudden increases in the load or developed tension in heart muscle. Often these phenomena are intermixed and may not be readily dissociable. They may be characterized as (1) homeometric autoregulation (9, 10), (2) the present findings, and (3) viscosity. The first phenomenon, which was described by Anrep (9) and elucidated by Sarnoff and Mitchell (10) in the intact heart, has been termed homeometric autoregulation. Following an abrupt and sustained increase in aortic pressure, an initial increase in left ventricular end-diastolic pressure was observed, with a subsequent fall in this pressure to an intermediate level despite the sustained increase in aortic pressure (10). This was taken as evidence of an increase in contractility associated with the increase in loading. In contrast, in the present study, after an abrupt increase in tension development, the first isometric contraction showed the greatest tension and rate of tension development, with a subsequent decline in isometric tension. Thus, the present effects are directionally opposite to those of homeometric autoregulation. However, a recent study (11) of the effects of increased loading on the contractility of isolated cat papillary muscle has demonstrated effects directionally similar to the phenomenon termed homeometric autoregulation and directionally opposite to those of the present study. These differences (11) may be due to a lower temperature (25°C) and a higher frequency of stimulation (30/min) than were routinely used in the present study. Certainly the results in Figure 6 indicate that the phenomenon described in our study was substantially reduced at lower temperatures, although an opposite effect was never observed.

An important question posed by the present study is whether the changes observed between the first and stable isometric contractions represent a real change in contractility or the manifestation of a mechanical property of heart muscle, i.e., a viscous element. Were this phenomenon to represent a change in contractility, it would probably be mediated by an increased availability of intracellular calcium during an isotonic contraction, with a subsequent slow decline as the mode of contraction was abruptly changed to isometric. Thus, the prolongation of contraction with heightened tension development is what one would anticipate from a decrease in the intracellular binding and removal of calcium. Further, the time course of the decline in isometric tension resembles the decay of contractility following potentiation with paired electrical stimulation, which is known to be mediated by changes in intracellular calcium (12). In addition, in our laboratory, rat papillary muscles have not demonstrated this phenomenon and have not shown an increase in tension development with paired stimulation at a frequency of 6/min (unpublished observations). If this phenomenon were simply mechanical, it presumably would also be present in rat muscle. If it is mediated by changes in intracellular calcium, however, it presumably would not be present in rat papillary muscle stimulated at 6/min, since paired stimulation (which acts by increasing available calcium) also does not increase developed tension. Furthermore, the delay in relaxation during the first isometric contraction is similar to that observed follow-
ing an increase in the calcium concentration bathing the muscle (13). Lastly, the increase in $V_{\text{max}}$ (Fig. 9) is characteristic of an increase in contractility (4, 5).

However, it should be noted that this supposed increase in the contractile state is somewhat unusual in character since an increase induced by calcium or other positive inotropic influences is invariably associated with a decrease in the time to peak tension (4, 5), while the first isometric contraction in the present study always evidenced a prolonged time to peak tension. Furthermore, this phenomenon was present and only slightly diminished after maximum potentiation of tension following increased calcium, paired stimulation, or the addition of isoproterenol (Fig. 7). Generally, significant additional potentiation of contractility is not produced when the muscle is already very close to its maximum force development (7). Thus, one may reasonably question whether this phenomenon may simply be explained by an increase in contractility.

An alternative explanation for these phenomena may be the presence of a viscous element which can be represented in mechanical terms by a dashpot and parallel spring. This is the third type of load-dependent phenomenon that has been described. Thus, during active contraction, small decreases in resting (diastolic) tension at constant muscle length have been demonstrated whenever force of contraction is augmented. Since this small fall in resting tension depends only on the increase in load, it has been attributed to a viscous element arranged in series with the contractile elements (14).

In Figure 12, one arrangement of the three-component mechanical model proposed by A. V. Hill and associates (1) is diagrammed. At rest, the contractile element (CE) is presumed to be freely extensible, and, with activation, generates force and shortens. The contractile element is arranged in series with an elastic element (SE), which has the properties of an exponential spring (9). In the particular arrangement of the model seen in Figure 12, the parallel elastic element (PE) contributes to resting tension (8). Possible locations for viscous elements (VE) are represented by VE 1, 2, and 3. Previous studies (14, 15) have offered evidence for a viscous element in series with the muscle (VE 3). Assuming such an element, VE 3 would have only resting tension across it during the isotonic mode of contraction. During the first isometric beat, tension increases, VE 3 would be elongated, and the contractile element would shorten concomitantly. This would lead to a reduction of tension in subsequent contractions. However, were this an adequate explanation for the data, increasing muscle
length alone would restore the original length of the contractile element and return tension to the same level as that of the first isometric contraction. This is not so, as is shown in the experiment of Figure 13. As indicated by the tension trace at high gain, there was a slight fall in resting tension when isometric tension was developed, which is a predicted effect for a series viscous element. Indeed, in 14 muscles with an average preload of 0.75 g, there was a decline in resting tension during the transition from isotonic to isometric contractions which averaged 0.04 g (5.3%). Following stabilization of the isometric tension, muscle length and preload were increased (arrows) so as to restore resting tension to its original level. Nevertheless, this increase in the preload to its initial level augmented developed tension only slightly, and to a level which was far below the level of the first isometric contraction. In fact, if initial resting tension was greater than 30% of that at the apex of the length-tension curve, the first isometric beat developed a greater tension than could be produced by any further increments in muscle length (Fig. 3). Thus, although support for a series viscous element (VE) was gained from the fall in resting tension associated with increasing load, the existence of such a series viscous element cannot by itself explain the results of the present study.

The use of a metal clip on the muscle-tendon junction virtually eliminated equipment viscosity (Fig. 11) as a possible explanation for the slight fall in resting tension which has been observed. However, the bottom of the muscle was held firmly in a spring-loaded lucite clip, which damages a very short segment of the muscle and might account for the small viscous change observed and suggest the presence of a series viscous element (VE).

The role of other viscous elements also warrants comment. If there were a viscous element VE, it might participate in the stress relaxation associated with a sudden increase in muscle length and resting tension; however, it could not produce changes in developed tension since it is not in series with the contractile element. However, a viscous element (VE) in close association with the contractile element might help to explain the changes in peak tension observed in the present study. Thus, during the first isometric beat, VE would be subjected to stress and would slowly elongate. The contractile element would then shorten in a parallel manner. Accordingly, there would be a reduction in the tension developed by subsequent beats. However, when one attempts to restore the contractile element to its original length by increasing muscle length, the contractile element would increase in length in proportion to the increase in length of the parallel elastic element. In this particular arrangement of the three-component model, the parallel elastic element would be a very stiff spring (8) so that it might not be possible to restore the contractile element to its original length, despite extension of the muscle to the top of the length-tension curve. However, it is important to note that such a viscous element cannot readily explain the prolongation of contraction and delay in relaxation which has been observed.

In summary, an abrupt load imposed on the isolated cat papillary muscle by suddenly changing the mode of contraction from...
isotonic to isometric has revealed a heightened contractile state during isotonic contraction. This phenomenon might be explained by a decreased rate of removal of intracellular calcium. Further, some aspects of the phenomena also suggest the existence of a viscous element in close association with the contractile element.

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