Active Length-Tension Relations Compared in Isometric, Afterloaded and Isotonic Contractions of Cat Papillary Muscle

THEIR DEPENDENCE ON INOTROPIC STATE

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With the Technical Assistance of Peter Burrows

ABSTRACT

Isometric, afterloaded and isotonic contractions of cat right ventricular papillary muscle were studied in vitro at 30°C or 37°C. The length to which muscle contracted under a given total load varied directly with initial muscle length. The discrepancies between contracted muscle lengths depended upon load and the inotropic state of the muscle. In control contractions at 12/min, the maximum discrepancy occurred at a load of 0.5 g or 1 g and averaged 0.47 ± 0.04 (mm) while after acetylstrophanthidin (0.75 μg/ml) the maximum discrepancy occurred between 3- and 5-g load and averaged 0.62 ± 0.07 mm (P < 0.001). At low loads acetylstrophanthidin decreased the discrepancy at 0.5-g load from 0.41 ± 0.08 mm to 0.22 ± 0.05 mm (P < 0.001). Acetylstrophanthidin increased developed tension at optimum muscle length by 71 ± 23% (P < 0.001). Increasing frequency of contraction from 12/min to 60/min induced greater decrease in time to peak tension than occurred with acetylstrophanthidin (30% vs. 15%) but produced little change in either isotonic or isometric contracted length-tension relations. Since the differences between contracted length-tension relations were not influenced by abbreviation of active state induced by frequency changes, it is unlikely that the lesser abbreviation with acetylstrophanthidin was responsible for the changes it produced. Rather, they may be related to the intensity and duration of active state reflecting increased energy release in the contractile process.

ADDITIONAL KEY WORDS cardiac muscle shortening treppe contractile state force-velocity active state acetylstrophanthidin initial length frequency of contraction

Some evidence suggests that the length-tension relation of contracted cardiac muscle is essentially independent of the initial resting muscle length and the amount of shortening during contraction. Other evidence suggests that the contracted muscle length depends on the nature of loading in the prior contraction; when muscle contracts isotonically against a load or tension, it fails to shorten to the length from which isometric contractions produce the same tension; and at a given load, contracted muscle length varies directly with initial muscle length. The last view is supported by the finding that the ejecting frog ventricle does not shorten to the isovolumic pressure-volume relation when examined over a wide range of filling (1, 2). This view is also supported by Rosenblueth et al. (3) as a result of a study in which dog's heart was made to develop tension and shorten through attachment to an external support. The contention that contracted muscle length depends little on initial muscle length has been supported by observations on cat right ventricular papillary muscle in vitro (4, 5), on the dog's isolated...
beats (6,7), and more recently by a study in the intact dog in which the volume-tension relation reached at end-ejection approximated the volume-tension relation of isovolumic contractions (8).

In the present study of right ventricular papillary muscle of cats, the length to which muscle shortened under a given load was found to vary directly with initial muscle length. The differences between contracted muscle lengths were shown to depend not only on initial muscle lengths and the load at which contractions were examined but also on the isotropic state of the muscle. Two qualitatively different positive isotropic interventions were studied: the one, addition of acetylstrophanthidin, produced a marked increase in both absolute tension development and velocity-related variables (9), and the other, increasing frequency of contraction, affected predominantly velocity (10). Both interventions decrease the time to peak tension and so reflect a decrease in duration of active state (11). From these studies it has been concluded that decreased duration of active state per se does not change the differences between contracted muscle lengths in isometric, afterloaded, and isotonic contractions at a given load. Rather, the changes observed with acetylstrophanthidin may be due to changes in the intensity and duration of active state reflected by the changes in active length-tension relations.

Methods

Right ventricular papillary muscles were rapidly removed from cats (1.3 to 3.2 kg) anesthetized with sodium pentobarbital (30 mg/kg ip). The length of the muscles averaged 6.9 ± 1.4 (m) mm and their cross-sectional area 0.96 ± 0.23 mm² at the length at which tension development was maximum (optimum length). The muscle was placed in a water-jacketed glass bath containing Krebs-Ringer solution bubbled with 95% O₂, 5% CO₂ (10) and maintained at a constant temperature of 30°C (37°C in these experiments) by a Tecum Tempunit TU8 unit. The non-tendinous end of the muscle was secured by braided non-corpillarized silk (Ethicon 0000) to a magnesium lever of ratio 20 to 1. Displacement was measured by a rotary variable differential transducer (Schaeft Engineering Model H 14 BSS), which formed the fulcrum of the lever. The muscles were stimulated through platinum electrodes placed parallel to the muscle by square wave pulses of 7 msec duration and voltage just above threshold, delivered from a Nihon Kohden Laboratory stimulator (Model MSE-3). Recordings were made on a multichannel Sanborn direct writing recorder (7700 Series).

Appropriate mechanical stops permitted the study of isometric, afterloaded, and purely isotonic contractions. Each muscle was first allowed to contract with 0.5-g preload at half, and subsequently full, isometric tension for 1 hour. Three properties of the muscle were then examined:

1. The force-velocity relation at 0.5-g preload, that is, the inverse relation between total load (constant preload + variable afterload) and the greatest velocity at which the muscle shortened against that load. Velocity was obtained from the maximum slope of the shortening recording and was also obtained continuously with an R-C differentiating circuit. The greatest velocity of shortening against 0.5-g preload alone, without afterload, is termed Vmax and the maximum rate of isometric tension development at 0.5-g preload (dT/dt)max. Time to peak tension (TTPT) was measured from the stimulus artifact to peak tension.

2. The passive and active isometric length-tension relations obtained by increasing muscle length, with the muscle contracting isometrically, up to the length from which maximum tension (DTmax) was actively developed (optimum length). The terms force, tension, and load are used interchangeably according to common usage, for example, force-velocity and length-tension, and are expressed in grams.

3. The initial and contracted lengths and amount of shortening of the muscle contracting under constant total load while initial resting muscle length was increased by the micrometer stop from a length at which the muscle failed to move the load to a length at which it was contracting isometrically, or until the optimum length was reached. A total load of 0.5 g was examined, then 1 g, 3 g, increasing by 1-g increments until shortening did not occur and only isometric contractions were possible at the optimum length.

At high total loads, contractions which just fail to move the load are isometric. At low total loads, they are not purely isometric but initially isometric against zero load and then isometric. The term isometric is used to include these contractions.

The equipment exhibited a certain compliance.
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with both rapid and slow viscous components. A length of silk knotted at its lower end was placed so as to simulate the experimental system without a muscle, and the compliance of seven such systems was measured. Rapid loading with 1 g resulted in an increase in length over the next 60 seconds of 0.04 ± 0.01 (SD) mm, of which all but 0.008 ± 0.004 mm occurred within 200 msec. The corresponding figures for 4 g were 0.17 ± 0.02 mm and 0.08 ± 0.02 mm. Interpretation of the results of contractions at constant total loads would be influenced by the slow viscous component of this compliance but not importantly by the rapid component.

All 27 muscles were examined initially at a contraction frequency of 12/min. Subsequently, in some experiments, the influence of acetyl-strophanthidin,1 0.75 µg/ml (five experiments) or of higher frequencies of contraction, 30/min and 60/min (seven experiments), was examined. Levels of significance were evaluated by Student's t-test.

Results

With a given total load, the length at which the contracting muscle just failed to move the load was determined (point a in Fig. 1). With total load held constant, the initial or resting muscle length was then progressively increased until the muscle was contracting isotonically, shortening from b to c (Fig. 1), or alternatively, with high loads until the optimum muscle length, as determined from the preceding length-tension relation, had been reached (b' in Fig. 1C).

Control Contractions at 12 per Minute.—Each increment in initial muscle length at constant total load resulted not only in a greater amount of shortening during contraction but also in a longer, fully contracted muscle length (Fig. 1). The magnitude of the maximum discrepancy between contracted muscle lengths at any one total load, represented by ac (Fig. 1), depended on the level of total load at which the contractions were examined. In 25 of 27 experiments (including all three in which the temperature was 37°C), ac was greatest at a small load of 0.5 g or 1 g and decreased at higher loads (Figs. 1 and 3); in two experiments ac was slightly greater at 2 g than at 1 g. This finding was not an artifact imposed by the experimental protocol, whereby initial muscle length was limited at higher total loads, since a load of 2 g was always examined isotonically and a load of 3 g usually so. The maximum value of ac when expressed as a percent of the corresponding potential isotonic shortening, i.e., ac/ab% averaged

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**FIGURE 1**

Recordings at three levels of total load, 1.0 g (A), 3.0 g (B), and 5.0 g (C) while initial muscle length was increased stepwise from the length at which the contracting muscle just failed to move the load (point a) to the length from which it contracted isotonically (b in A and B) or to the optimum length (b' in C).
Often this did not coincide precisely with the maximum value of \( ac/ab \), which averaged \( 31 \pm 5\% \), since at higher total loads \( ac \) tended to decrease less than \( ab \).

**Acetylstrophanthidin**—The changes induced in force and velocity by acetylstrophanthidin \((0.75 \mu g/ml)\) are included in Table 1. Figure 2 shows typical changes induced in contractions at constant total loads, \(0.5 \) g (Fig. 2A) and \(4 \) g (Fig. 2B). In the experiment shown, \( ac \) at \(0.5\)-g load was \(0.33 \) mm; at \(1\) g it was \(0.47 \) mm, but then decreased to \(0.19 \) mm at \(4\) g (which was examined isotonically). In contrast, after acetylstrophanthidin, \( ac \) was maximal at \(4\)-g load, \(0.59 \) mm, but was only \(0.19 \) mm at \(0.5\)-g load. A typical plot of contracted muscle length against load (tension) is shown in Figure 3, and the results from five experiments are summarized in Table 2. Acetylstrophanthidin increased the maximum discrepancy between contracted muscle lengths, \( ac \), which also occurred at a higher load (Table 2, Fig. 3). Although this represented the same proportion of potential isotonic shortening, \( ab \), the maximum value of \( ac/ab \), which occurred at a still higher load, was increased. These values of \( ac \) and \( ac/ab \) were obtained from afterloaded contractions (Fig. 3) since isotonic contractions were not examined at the high loads at which \( ac \) and
### Table 1

<table>
<thead>
<tr>
<th>Intervention</th>
<th>$V_{max}$ % change</th>
<th>$\frac{d}{dt}T/\Delta t$ % change</th>
<th>TTPT % change</th>
<th>DT % change</th>
<th>DT_{max} % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylstrophanthinid</td>
<td>67 ± 20</td>
<td>113 ± 26</td>
<td>-15 ± 3</td>
<td>57 ± 22</td>
<td>71 ± 23</td>
</tr>
<tr>
<td>Frequency 12 → 30/min</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>P</td>
<td>45 ± 10</td>
<td>44 ± 20</td>
<td>-11 ± 7</td>
<td>17 ± 9</td>
<td>5 ± 11</td>
</tr>
<tr>
<td>Frequency 12 → 60/min</td>
<td>&lt; 0.001</td>
<td>&lt; 0.005</td>
<td>&lt; 0.001</td>
<td>&lt; 0.005</td>
<td>NS</td>
</tr>
<tr>
<td>Acetylstrophanthinid vs frequency 12 → 60/min</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are means ± 1 SD. $V_{max}$ = maximum velocity at 0.5-g load; $\frac{d}{dt}T/\Delta t$ = maximum rate of isometric tension development at 0.5-g preload; TTPT = time to peak tension; DT = developed isometric tension at 0.5-g preload; DT_{max} = developed isometric tension at optimum length.

Increasing Frequency of Contraction.—The effects of increasing frequency of contraction are included in Table 1, and the effects of changing from 12 to 60/min are compared to those induced by acetylstrophanthinid. The

$ac/ab$ were maximum, and hence the changes induced by acetylstrophanthinid were almost certainly underestimated. At light loads of 0.5 g and 1 g, $ac$ was decreased by acetylstrophanthinid (Table 2, Figs. 2 and 3).
Effect of Acetylestrophanthidin on Contractions at Constant Total Load

<table>
<thead>
<tr>
<th>Load at 0.5-g preload (g)</th>
<th>0.5 or 1.0</th>
<th>3.0 to 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max ac at 0.5-g preload (mm)</td>
<td>0.47 ± 0.04</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>Max ab (%)</td>
<td>20 ± 4</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>Max ac/ab (%)</td>
<td>0.41 ± 0.08</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>ac at 60/min (mm)</td>
<td>0.41 ± 0.08</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are means ± 1 sd. ac = difference between contracted muscle lengths of isometric and isotonic contractions; ab = potential isotonic shortening (see text).

Increases in maximum velocity at 0.5-g load ($V_{max}$) and in the maximum rate of tension development ($DT/dt_{max}$) were similar in response to the two interventions, but the decrease in time to peak tension ($TTPT$) was significantly greater with the change in frequency. Maximum tension development ($DT_{max}$) was unchanged by increasing frequency to 60/min and tension developed from 0.5-g preload was slightly increased, responses significantly different from those to acetylestrophanthidin. In contractions examined at constant total loads, maximum ac increased only 0.024 ± 0.032 mm ($P < 0.1$), which was significantly less than with acetylstrophanthidin ($P < 0.005$), while no change was induced in ac/ab.

Discussion

In this study, the relation between contracted muscle length and load or tension depended on the nature of loading during the prior contraction. With constant total load, the greater the initial resting muscle length and the more shortening that the muscle underwent against the load, the longer the contracted muscle length, so that the greatest difference in contracted lengths was between isometric and purely isotonic contractions. The magnitude of this discrepancy depended on the level of load at which the contractions were examined, was considerably influenced by acetylstrophanthidin, but was little affected by changing frequency of contraction. As pointed out in skeletal muscle studies (12), "isometric" contractions under small total loads are not solely isometric but are initially isometric under zero load and then isometric. This does not invalidate the conclusions but has been invoked to explain the absence of a difference between contracted lengths of isometric and isotonic contractions at low loads. In the present study it was under such light loads that the greatest discrepancy occurred, at least in the absence of a positive inotropic intervention.

It seems unlikely that equipment compliance importantly influenced the results. Compliance having a rapid time response would be a constant in comparing contracted muscle lengths at any one total load. However, a slow "viscous" component of equipment extensibility would make contracted muscle lengths appear greater in contractions originating from higher resting tensions if the equipment had time to "stretch out." The measurements made suggest that this could account for only a small percent of the discrepancy found between contracted muscle lengths at low loads, as much as 25% of the discrepancy at high loads under control conditions, and about 10% at high loads after acetylstrophanthidin. 2

Extension of viscous components in series with the contractile elements of cardiac muscle (13, 14) could have been responsible for the observed differences in contracted muscle lengths even if the contractile elements

2If all equipment compliance were relevant, it could account for 10% of the discrepancy at low loads, all of it at high loads under control conditions where the measured discrepancy was small, and 25% at high loads after acetylstrophanthidin, but this does not seem to be so.
shortened to the same length under constant load. The discrepancies would then be expected to have increased with increasing load, but this occurred only in the presence of acetylstrophanthidin; otherwise, the discrepancies decreased at high loads so that viscous effects either in the equipment or in the muscle itself are unlikely to be primarily responsible.

The alternative hypothesis is that the contracted length-tension relations of contractile elements depend to some extent on initial muscle length. Nevertheless, it is impressive that there is such a good approximation, as recently emphasized by studies in the intact heart (5) and in vitro by the observations of Sonnenblick that the force-velocity relation of cat papillary muscle studied at 21°C depends on instantaneous muscle length and that fully contracted muscle length is little affected by initial length: (5). These observations suggest that the rate of energy conversion to contractile activity (intensity of the active state) is partly regulated by instantaneous muscle length and that increasing initial muscle length increases the duration of the active state (5, 8). However, in vitro at temperatures of 30°C or above, Sonnenblick also found that contracted muscle length became dependent on initial length, an effect he attributed to abbreviation of the active state, since contractions departed from a common force-velocity-length relation only toward the end of contraction (5, 15). Instantaneous force-velocity-length relations were not examined in the present study, but, accepting that it is the relatively short duration of the active state in a single cardiac muscle twitch (11, 16) which limits afterloaded and isotonic contractions somewhat more than it does isometric contractions, further abbreviation of the active state might have been expected to accentuate the difference between contracted muscle lengths in these different contractions. Yet such an effect of duration of the active state per se was not observed. Increasing frequency of contraction from 12/min to 60/min with considerable (30%) decrease in time to peak tension, reflecting a decrease in the duration of the active state (11), did not greatly influence the difference between contracted muscle lengths at a given load. Rather, the contracted length-tension relations of both isometric and isotonic contractions failed to change very appreciably despite the increase in rate of tension development and rate of shortening. On the other hand, acetylstrophanthidin, which produced a significantly smaller (15%) decrease in time to peak tension but a marked increase in isometric tension development, also produced a large increase in the difference between contracted muscle lengths of isometric, afterloaded, and isotonic contractions at high loads. These observations suggest that the latter effect is not directly attributable to decrease in duration of active state but rather that it may be related to the change in isometric tension development and shift in contracted length-tension relations which reflect an increase in intensity and duration of the active state. At low loads, the discrepancies between contracted muscle lengths were actually decreased by acetylstrophanthidin; this was contrary to the prediction that at these loads, under which isotonic contractions shorten furthest, positive inotropic interventions would particularly curtail afterloaded and isotonic contractions by decreasing duration of active state.

Precise interpretation of the results in terms of myocardial energetics cannot be given, but the following descriptive analysis is presented. Under the conditions of this study, the greater energy available to the contractile mechanism as a result of increasing muscle length (Starling effect) was not quite sufficient to maintain contracted length-tension relations constant. Presumably the discrepancy depends on the energy available to the contractile process and the energy requirements in different types of contractions. Change in position of the isometric contracted length-tension relation produced by positive inotropic influences like acetylstrophanthidin, which necessarily reflects an increase in the intensity and duration of the active state and an increase in the energy expressed in contractile activity, produces effects as though the tension...
scale in Figure 3 were expanded. The tension developed by isometric contractions, the shortening by isotonic contractions, the discrepancy between contracted muscle lengths at most loads, and the load at which this discrepancy is greatest, are all increased. At low loads, at which the discrepancy is maximum in the control state, the discrepancy becomes less because that absolute level of load is less relative to the increased performance of the muscle and much below the load at which the discrepancy is now maximum.

Recently (8) the left ventricle of the intact dog was found to eject to an average of 2 ml (or approximately 10% of stroke volume) from the volume-tension relation of isovolumic contractions, and, although in three of nine animals ejection seemed to be decidedly curtailed (by a volume equivalent to 21%, 28% and 37% of stroke volume), overall there was not a significant difference between contracted volume-tension relations. Similar observations on the left ventricle have been made previously (6, 7) and the question of how they can be reconciled with the present results is posed. In Figure 4, a contraction of the intact left ventricle, quite heavily and variably afterloaded, is represented schematically by ABCD and is superimposed on the results of an in-vitro experiment. In this experiment, when the cat papillary muscle developed tension from A to B and shortened against a constant load equivalent to peak left ventricular tension, it reached F, failing to reach the isometric length-tension relation by 17% of the amount it had shortened. When the muscle developed tension from A to E and shortened against a load equivalent to the load at end-ejection it reached C, 33% (or alternatively 25% of potential shortening) from the isometric length-tension relation. An exact analogy with left ventricular contraction cannot be drawn, however, for the following reasons:

1. The contractile state of muscle in vitro is probably depressed compared to that in vivo, and the present study has shown that the inotropic state affects the differences between contracted muscle lengths. Also, since active tension development is greater relative to resting tension in the intact ventricle, the
length-tension diagrams are not precisely comparable.

2. Factors other than muscle function influence the nature and probably the extent of left ventricular ejection. The unloading which occurs throughout most of ejection (8), the effect of instantaneous impedance (17), the influence of momentum, acceleration, and deceleration of blood (18), and the complexity of left ventricular architecture and contraction (19).

Furthermore, in the intact ventricle isovolumic and ejecting contractions have been compared. Because of shape changes during isovolumic contraction, the comparisons are presumably between very heavily afterloaded, but not isometric, contractions and less heavily afterloaded, ejecting contractions. The present study predicts that the differences would be smaller than between afterloaded, ejecting beats and isometric contractions, if these could be compared. Also it should be noted that, because of three-dimensional geometry, given changes in a linear variable such as muscle length at small dimensions near end-ejection are associated with smaller changes in volume than they are at large dimensions; a percent discrepancy in contracted muscle length is associated with a smaller percent discrepancy in ejected volume. Previously (8) it was concluded that the behavior of the intact ventricle was consistent with that already shown in isolated cardiac muscle in which, under certain conditions, the relation between contracted muscle length and tension was largely independent of initial muscle length (4, 5). Under the conditions of the present study, this relation was partly dependent on initial muscle length, but the observations in this in-vitro study and in in-vivo studies are not in great disagreement. Although the variables already discussed complicate the mechanics of ventricular ejection, in the light of the present study it is quite possible that the average difference found (8) between volume-tension relations of ejecting and isovolumic contractions was a real difference. Because the difference was small and measurements in vivo are difficult, it was not observed consistently enough to reach statistical significance.

The present study of papillary muscle in vitro has allowed the accurate quantification of the influence of initial muscle length on constructed length and has characterized the dependence of this effect on inotropic state.

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