Comparison of the Effects of Different Inotropic Interventions on Force, Velocity, and Power in Rabbit Myocardium

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To gain some insight into inotropic mechanisms, we compared the effects of several classes of inotropic interventions on the isometric twitch and force-velocity properties of isolated rabbit myocardium. Postextrasystolic potentiation was chosen as one of the interventions in the belief that its onset is so rapid that it would be unlikely to cause substantial chemical changes in the contractile proteins and that most of the effects would be due to changes in the level of activation. The effects of a digitalis analogue (acetylstrophanthidin), an adrenergic agent (isoproterenol), and a methylxanthine (caffeine) were then compared with those of postextrasystolic potentiation. The conditions were chosen so that each agent caused a twofold increase in twitch force. Acetylstrophanthidin and postextrasystolic potentiation caused twitch force to increase with only a slight (11%) decrease in time to peak force. Isoproterenol caused the peak of the twitch to occur substantially (40%) earlier with marked abbreviation of the twitch. Caffeine had the opposite effect: time to peak force was delayed (by 60%), and the twitch was markedly prolonged. In contrast to the marked differences in the time course of the twitch, there was no significant difference between the instantaneous force-velocity curves obtained with the different interventions. All four interventions caused maximum velocity to increase slightly (1-9%) and maximum power to increase only slightly more than twitch force (5-21%). All of the changes observed can be accounted for by changes in activation, either by an increase in the amount of calcium released into the myofilament space or by a change in the sensitivity of the myofilaments to calcium. There was no need to postulate direct changes in the contractile machinery to account for these results. (Circulation Research 1989;65:1161-1171)

It is well known that different inotropic interventions have different effects on the shape of the twitch contractions in isolated cardiac muscle.1 This raises the question of whether the different interventions alter the extent of muscle activation or whether they also change the contractile response of the activated myofilaments. For technical reasons, previous efforts to address this question have not given unimpeachable results. The studies were done with mechanical levers that produced sustained oscillations when the load was suddenly changed. To avoid these oscillations, the earliest studies used “afterloaded contractions” to obtain individual points on a force-velocity curve.2-4 The ends of the muscles were held at a fixed length during the onset of a twitch until the force in the muscle rose to the value of an afterload, determined by a weight hung from a lever attached to the muscle. After that time, the muscle shortened at a constant load determined by the weight. Either the initial or the maximum velocity was plotted as a function of load, and multiple points were obtained by using different afterloads in different contractions. These experiments were criticized because each point on the force-velocity curve was obtained at a different time in the twitch and at a different sarcomere length.5-7 It was generally recognized that the correct way to do the experiments was to release the muscle to different loads at the same time in the twitch and at a different sarcomere length.5-7 In this way, the level of activation and the amount of sarcomere shortening, caused by extension of the series elastic element as force developed, would always be the same at the onset of shortening. Until recently, attempts to make these types of experiments were not technically feasible. Large, poorly damped oscillation prevented measurement of short-
enishing velocity for many tens of milliseconds after the release, and there was some evidence that these oscillations inactivated the muscle, although there is the additional suggestion that this inactivation is caused by the rapid shortening associated with the quick release and not with the oscillations themselves. In one study of the effects of methylxanthines, the rapid lever movement was damped to reduce or eliminate oscillations, but the force-velocity measurements were not made until a substantial amount of shortening had occurred after a steady force was achieved.

To reexamine the question of the effect of inotropic agents on contractile capability, we have measured the force-velocity properties of rabbit papillary muscle with a servo system capable of completing critically damped force steps within 1-3 msec. This high speed was necessary to make measurements a few milliseconds after a release and, thereby, to minimize the cumulative effects of progressive shortening. Our previous experiments have shown that, even with these rapid steps, damped recoil of the series elastic elements and velocity transients preclude measurements earlier than 5–6 msec after the onset of the step and that velocity begins to decline almost immediately with the onset of shortening. Thus, there is only a brief period after a quick release when muscle shortening velocity can be used to assess the contractile capability at the time of the release. In the present study, we have measured velocity during a 6–10 msec period after the onset of the step.

In this study, we have compared the influence of several inotropic interventions on the isometric twitch contraction and on the instantaneous force-velocity relations of rabbit papillary muscle. The interventions chosen include isoproteorenol, which shortens twitch duration, caffeine, which lengthens twitch duration, a digitalis analogue (acetylstrophanthidin), which potentiates the twitch without greatly affecting its time course, and postextrasystolic potentiation. This last intervention was chosen because its effects are so rapid, within one beat, that there is little time for a metabolic or biochemical change to occur and its effects are believed to be due almost entirely to changes in calcium release. The influence of the other interventions on the force-velocity curves could thus be compared with those of postextrasystolic potentiation to determine whether their effects could be distinguished from simple increases in activation.

**Materials and Methods**

**Preparation**

Young adult New Zealand White rabbits weighing 2.0–2.5 kg were anesthetized with 100 mg ketamine and 40 mg xylazine, and their hearts were removed. Right ventricular papillary muscles or trabeculae having diameters less than 1 mm were dissected free and mounted in platinum foil clips. Hooks on the motor and force transducer were passed through holes in the clips to attach the muscles to the apparatus. Throughout the initial dissection procedure, the hearts and isolated muscles were kept at about 37° C. Once attached to the apparatus, the muscles were gradually cooled to room temperature (23°–25° C). In our hands, the technique of keeping the muscle warm during dissection and mounting was much more successful in yielding muscles that produced good force than the technique of cooling the hearts rapidly and performing the dissections near 0° C. Muscles that were dissected at room temperature or in the cold frequently shortened substantially during dissection and did not develop much tension.

At the end of the experiment, the muscles were removed from the apparatus, cut free of the clips, blotted dry, and weighed. Cross-sectional areas were determined as the weight divided by 1.05 times the length. The coefficient 1.05 g/cm³ was taken to be the density of the muscle.

**Solutions**

All experiments were done in a physiological salt solution containing (mM) NaCl 118, KCl 5, NaH₂PO₄ 1.2, NaHCO₃ 22.5, MgSO₄ 2, CaCl₂ 1, and HEPES 5, adjusted to pH 7.4 with NaOH after being equilibrated with 95% O₂-5% CO₂. During the experiments the solutions were kept in a reservoir where they were continuously perfused with the same gas. The solution was fed by gravity through a closed system of tubing and through the muscle chamber, which was also closed.

Inotropic agents were added to the solution either as dry powder (caffeine and acetylstrophanthidin) or as a ×50 concentrated solution (dl-isoproterenol); and pH was readjusted after equilibration with the gas. The final concentrations of these agents were 10 mM caffeine, 11 μM acetylstrophanthidin, and 4.5 μM isoproterenol.

**Apparatus**

The apparatus that has been described previously was modified only slightly. Briefly, a servo-controlled linear motor was used to adjust muscle length. The motor was held horizontally, and a horizontal length of stainless steel tubing was projected through a seal at one end of the horizontal muscle chamber. The chamber was 2 mm deep x 2 mm wide x 15 mm from the closed end of the trough where solution entered to the open end of the chamber, about 3 mm beyond the downstream end of the muscle. The chamber was covered by a glass plate, and fluid that was infused into the motor end was drawn away by suction at the other, open end. There was a vertical meniscus at the open, downstream end, and a hook from a 2–3 kHz photoelectric transducer projected through the vertical meniscus to the clip on one end of the muscle. The solution flowed through the trough at the rate of about 1 ml/min. The trough had a cross-sectional...
area of 4 mm² so that solution passed the muscle at a rate of about 4 mm/sec.

The motor could be controlled with a signal from its own internal position sensor (length control) or with a signal from the force transducer (force control). Switching between the two types of control was accomplished by a diode switching network. Digitized force and length records were obtained and analyzed with an IBM PC computer equipped with a Tecmar Labmaster board. The data collection and the timing of the experimental events were accomplished through this board with the SALT software package.

**Protocol**

Muscles were stimulated every 1.2–2 seconds, a stimulus interval slightly longer than that required to produce maximum isometric twitch force. In our early experiments (most of those with isoproterenol and acetylstrophanthidin), isotonic steps were applied every sixteen twitch; the intervening 15 twitches were used to let the muscle return to a steady state, and the time was used to store the data records on disk. With more sophisticated programming, the time between isotonic steps could be diminished, and every eighth twitch was studied with an isotonic contraction. When postextrasystolic potentiation was used, it was applied in alternate test contractions, that is, every thirty-second or sixteenth contraction. In this way, potentiated and nonpotentiated contractions were studied together.

After the preparation was mounted, a period of stabilization was allowed before data were collected. Once the muscles were stabilized, the length-tension relations were measured to define Lmax, the longest length at which the developed force–length relation had a positive slope. The muscle length was then set at 90% of this value, and all further measurements were made at this length. This length was chosen because rest tension was very small. The slow recording was used to determine the time-tension integral during the rise of force and the time required for the muscle to stabilize, the force-velocity measurements were not made until 20–30 minutes after the solution was changed. The effects of postextrasystolic potentiation were not measured in the presence of these agents because it had no additional potentiating effect in the presence of isoproterenol and caused a slight decrease in the presence of caffeine. Although it did further potentiate the twitches in the presence of acetylstrophanthidin, its effects are not described here. Similarly, the control experiments were not repeated after washout of the potentiators because, with isoproterenol and caffeine, the effects were not reversible. With isoproterenol, the potentiated force did not decline substantially over periods of 30–60 minutes after washout. With caffeine, the unpotentiated twitch force fell to baseline levels within a few minutes of washout, but the twitches were not potentiated by extrasystoles. Both normal and potentiated twitch force fell to baseline levels within 20–40 minutes after the washout of acetylstrophanthidin, but to keep the method of analysis consistent, data from these washout experiments are not included.

**Data Analysis**

The computer was programmed to collect data at two different rates. The whole twitch was recorded at intervals of 5 msec per conversion of both the length and tension signals. At the time of peak tension, the interval was decreased to 200 msec per conversion of each signal for 50 msec and then increased to the slower 5-msec interval to complete the recording of the twitch contraction (Figure 1). The slow recording was used to determine the time-tension integral during the rise of force and the maximum rate of rise of force. The isotonic force was determined from the fast records by averaging the force record over a 4-msec period from 6 to 10 msec after the onset of the step (between dotted

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FIGURE 1. Force and length recordings showing the time course of the whole twitch at a slow recording speed and the time course of isotonic shortening at a 25 times greater recording speed. Lmax, longest length at which the developed force-length relation has a positive slope. The transitions between fast and slow recording speeds are indicated by the interrupted vertical lines. The times over which force and velocity measurements were made (6–10 msec after the isotonic step) are indicated by the vertical dotted lines. Muscle length at Lmax was 3.2 mm; cross-sectional area was 0.51 mm².

The velocity was determined by fitting a linear least-squares regression to the length record over the same 4-msec period.

**Force-Velocity Parameters**

The force-velocity data obtained in this manner were fitted by a nonlinear least-squares (Newton-Gauss) procedure to the Hill equation:

\[
\text{Velocity} = \frac{c}{(\text{Force} + a)} - b
\]

where \(a\) and \(b\) are the force and velocity asymptotes, respectively, of a hyperbola and \(c\) is a constant. The parameters of the hyperbola \((a, b,\) and \(c)\) were then converted to the more physiological parameters \(P_o\) (isometric force), \(V_m\) (maximum velocity), and \(P_{Vm}\) (maximum power) by use of the equations:

\[
\begin{align*}
P_o &= \frac{c}{b} - a \\
V_m &= \frac{c}{a} - b \\
P_{Vm} &= ab + c - 2\sqrt{abc}
\end{align*}
\]

Typical force-velocity and force-power curves are shown in Figure 2. The power values were obtained by multiplying force times velocity.

Slightly different values for the three parameters could be obtained if the fits were done on the force-velocity curves or the force-power curves, as illustrated in Figure 2. When the fits were made to the force-velocity curves, as in Figure 2A, the curves passed very close to the low-load high-velocity points. These curves usually passed slightly below the intermediate points and slightly above the low-velocity points. When the fits were done in this manner, the extrapolated isometric force was usually 10–20% greater than peak twitch force, and the peak of the power curve was slightly less than the highest measured points (Figure 1D). This result probably occurred because the higher velocities had larger residuals (larger differences between the data points and the dotted lines) and the fitting procedure gave greater weight to these residuals.

When the fits were made to the force-power curve, as in Figure 2E, a slightly different result was obtained. The fitted curve lay very close to the peak

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**Figure 2.** Graphs showing force-velocity (upper panels) and force-power (lower panels) data fitted by three different methods. Panels A and D: Curves fitted to the force-velocity relation. Panels B and E: The curves are fitted to the same data expressed as the force-power relation. Panels C and F: The curves fitted to force-velocity relation weighted for power. Arrow in panel A indicates point derived from record in Figure 1.
TABLE 1. Contractile Properties of Isolated Muscles in the Control State Before Intervention

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Number of muscles</th>
<th>L_{max} (mm)</th>
<th>Cross-sectional area (mm²)</th>
<th>Rest/twitch force ratio</th>
<th>Twitch force (mN/mm²)</th>
<th>Time to peak (msec)</th>
<th>Maximum dP/dt (mN/mm²/sec)</th>
<th>Tension-time integral (mN-sec/mm²)</th>
<th>Maximum velocity (L_{max}/sec)</th>
<th>Maximum power (mW/g)</th>
<th>Relative maximum power (L_{max}/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postextrasystolic potentiation</td>
<td>12</td>
<td>4.22±0.43</td>
<td>0.048</td>
<td>16.5±0.017</td>
<td>284±67</td>
<td>105±38</td>
<td>2.46±0.72</td>
<td>2.31±1.70</td>
<td>4.65±0.29</td>
<td>0.72±1.70</td>
<td>4.05±1.70</td>
</tr>
<tr>
<td>Acetylstrophanthin</td>
<td>6</td>
<td>4.35±0.48</td>
<td>0.041</td>
<td>19.0±0.012</td>
<td>306±44</td>
<td>88±17</td>
<td>2.65±1.26</td>
<td>2.27±0.76</td>
<td>4.50±0.87</td>
<td>0.76±1.94</td>
<td>0.049</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>6</td>
<td>4.37±0.39</td>
<td>0.048</td>
<td>19.0±0.019</td>
<td>285±44</td>
<td>129±44</td>
<td>2.87±1.36</td>
<td>2.77±0.53</td>
<td>5.76±0.31</td>
<td>0.53±0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>Caffeine</td>
<td>5</td>
<td>3.94±0.59</td>
<td>0.049</td>
<td>15.7±0.020</td>
<td>250±40</td>
<td>162±82</td>
<td>2.03±1.22</td>
<td>2.64±0.46</td>
<td>4.86±0.32</td>
<td>0.46±1.96</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Values are mean±SEM. L_{max}, longest length at which the developed force-length relation has a positive slope. Relative maximum power is the maximum power divided by the isometric force.

Results

The isometric twitch and force-velocity parameters for each set of control contractions are listed in Table 1. The effects of each intervention on these parameters is described separately for isometric twitch in Table 2 and for force-velocity parameters in Table 3.

Isometric Twitches

The effects of the four different inotropic interventions are illustrated in Figure 3. Each intervention caused a twofold increase in twitch force, but the time course of the twitch was affected in different ways. Postextrasystolic potentiation (Figure 3A) and acetylstrophanthin (Figure 3B) caused twitch force to increase with only a slight reduction in the time to peak force. With these two interventions, the general shape of the twitch was little altered, such that the maximum rate of rise of force (dP/dt_{max}) and the time-tension integral during the rise of force scaled in approximate proportion to the peak force (Table 1). By contrast, isoproterenol caused a substantial reduction in time to peak force (Figure 3C) so that the rate of rise of force was increased to a much greater extent than peak force and the time-tension integral was only slightly increased. Caffeine had the opposite effect (Figure 3D). Table 2 compares the mean effects of the four interventions on these three commonly used indexes of contractility for all muscles in the series.

The effects of the first three interventions were fairly straightforward and were measured simply after the addition of the intervention. The effects of caffeine, on the other hand, were more dependent on stimulation frequency. When caffeine was given to muscles stimulated at the usual interval of 1.2-2.0 seconds, the addition of caffeine caused an initial large increase in twitch force, with a prolonged twitch, as shown in Figure 3D. This initial effect usually did not last sufficiently long to measure the force-velocity curves. Within a few min-

TABLE 2. Percent Change in Isometric Twitch Parameters With Different Inotropic Interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Number of muscles</th>
<th>Twitch force (%)</th>
<th>Time to peak (%)</th>
<th>Maximum dP/dt (%)</th>
<th>Tension-time integral (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postextrasystolic potentiation</td>
<td>12</td>
<td>140±49</td>
<td>-11±71</td>
<td>194±87</td>
<td>120±69</td>
</tr>
<tr>
<td>Acetylstrophanthin</td>
<td>6</td>
<td>101±39</td>
<td>-11±4</td>
<td>159±39</td>
<td>108±43</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>6</td>
<td>113±29</td>
<td>-40±11</td>
<td>272±79</td>
<td>22±30</td>
</tr>
<tr>
<td>Caffeine</td>
<td>5</td>
<td>78±25</td>
<td>55±41</td>
<td>2±8</td>
<td>134±101</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

TABLE 3. Percent Change in Force-Velocity Properties With Different Inotropic Interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Number of muscles</th>
<th>Twitch force (%)</th>
<th>Maximum velocity (L_{max}/sec)</th>
<th>Maximum power (mW/g)</th>
<th>Relative maximum power (L_{max}/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postextrasystolic potentiation</td>
<td>12</td>
<td>140±49</td>
<td>9±19</td>
<td>172±60</td>
<td>13±5</td>
</tr>
<tr>
<td>Acetylstrophanthin</td>
<td>6</td>
<td>101±4</td>
<td>5±24</td>
<td>115±68</td>
<td>5±17</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>6</td>
<td>113±8</td>
<td>1±20</td>
<td>157±36</td>
<td>21±19</td>
</tr>
<tr>
<td>Caffeine</td>
<td>5</td>
<td>78±5</td>
<td>2±8</td>
<td>124±42</td>
<td>19±12</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
FIGURE 3. Graphs showing comparison of the effects of four interventions on the time course of the twitch. In each panel the force has been divided by the peak force in the control twitch (ref).

utes, force fell to about 1.5 times control, and the time to peak fell to near control levels. The effects described here were obtained in a later series of experiments in which the stimulation interval was increased to 2.0–2.5 seconds. This slowing of the stimulation rate caused the control twitch force to fall, but it also allowed the caffeine-mediated increase of the force and the prolongation of time to peak force to last for as long as a half hour, which was much longer than was necessary to collect the data for a force-velocity curve.

Force-Velocity Relations

The effects of the four interventions on the instantaneous force-velocity relations were measured to compare the influence of these agents on the contractile machinery. Typical quick-release force and length records from control and postextrasystolic records are superimposed in Figure 4. The force in the potentiated contraction is 2.2 times that of the control throughout the record, but the relative forces, that is, the ratio of the isotonic load to the isometric force immediately before the step, are almost identical for the two. The length records show an initial rapid shortening that is substantially larger for the potentiated contraction. This early rapid shortening is attributed to recoil of elastic elements in series with the contractile elements, mainly at the ends of the muscle. This shortening is expected to be larger in the potentiated contraction because the drop in absolute force during the step is larger. Once the force step is complete, the subsequent shortening is similar in the two contractions.

Quantitative measurements of force and velocity were made over a 4-msec period between 6 and 10 msec after the release (between the dotted lines in Figure 4), and the force-velocity points obtained at different relative loads in different contractions are plotted in Figure 5. Figure 5A shows the velocity plotted as a function of the absolute force in control and potentiated contractions. To show how the curves scale, velocity is plotted as a function of relative force in Figure 5B. The plots nearly superimpose. The extent to which the curves coincide after scaling the forces will depend to some extent on the maximum velocity. If the maximum velocities are not the same, the curves will not superimpose exactly. In the plots of Figure 5, the maximum velocity for the potentiated contractions is slightly

FIGURE 4. Force and length recordings superimposed for control and postextrasystolic potentiated twitches. \( L_{\text{max}} \), longest length at which the developed force-length relation has a positive slope. The step to the isotonic load occurred 40 msec earlier in potentiated record, and the record has been shifted horizontally so that the times of the tension steps coincide. Muscle length was 4.3 mm; cross-sectional area was 0.71 mm².
higher, as are the remainder of the velocity points. In general, a slight (1–9%) increase in maximum velocity was obtained with all four interventions (Table 3). To know whether the velocities at intermediate loads were increased, it would be necessary to measure velocity at some intermediate reference load. The relative maximum power (maximum power divided by the isometric force) provides a convenient reference point in the middle of the curve. 22 As with maximum velocity, there is a slight increase in the relative maximum power in the example shown (Figure 5D) and in the curves obtained with all interventions (Table 3). Although slight (5–21%), these differences are statistically significant with three of the four interventions and suggest that the velocities at intermediate relative loads are definitely, but not substantially, increased.

Comparison of the Effects of Different Interventions

Table 3 compares the three parameters of the force-velocity curves for the different interventions. All four interventions caused an approximately twofold increase in the isometric twitch force. The small increases in maximum velocities were not significant and not significantly different from each other. The increases in relative maximum power were significant at the p < 0.05 level with three of the four interventions, but none of the increases were significantly different from each other.

Discussion

The effects of the inotropic agents on twitch contractions in rabbit myocardium described here are similar to those described earlier for cat papillary muscle. The main point to be made from the observations of isometric twitches is that the ranking of inotropic effectiveness depends very strongly on the index used to measure contractility. If peak twitch force were used to assess the changes, all four interventions would have been regarded as being nearly equally effective. If the maximum rate of force development is used, isoproterenol is much more effective, and caffeine is much less so than the others. The reverse order is obtained with the time-tension integral; caffeine is the most effective, and isoproterenol has only a slight positive effect. These data show that "contractility" can be strongly dependent on the way it is defined.

The similarity of the effects of all agents on the force-velocity relation is in marked contrast to the very different effects of the agents on the time course of the twitch. These similarities and differences place some limits on the possible sites of action of the various interventions. These sites of action can be divided into three broad categories: 1) calcium release into the myofilament space, 2) calcium binding to the myofilament, and 3) the contractile response of the activated myofilaments. Each of these will be discussed separately.

Calcium Release

Calcium release has been measured optically by injecting the calcium-sensitive bioluminescent protein aequorin into cells. All four of the agents studied have been reported to increase the size of the calcium transient after stimulation of cardiac muscle although the effect of caffeine is variable and, in the concentration used here, causes a decrease. In the control state, the optical signal
peaks very early in the contraction and declines to only slightly higher than rest levels shortly after twitch force reaches its peak. There is a substantial lag between the time course of the free calcium signal and the time course of twitch force. The four interventions studied here cause changes in the time course of the calcium signal that parallel to some extent the time course of twitch tension described here. Increases in stimulus rate and acetylcholine have the same effect on calcium concentration in the myofilament space. The optical signal to reach an earlier and higher peak than in the control state. By contrast, caffeine causes a prolonged release of calcium, with the optical signal reaching a higher peak much later than in the control twitches, and at the concentrations used here, the peak is often reduced.

For two of the interventions studied, the changes seen in the time course of the twitch can be explained by the observed changes in calcium release. Acetylcholine and postextrasystolic potentiation cause an increase in the amount of calcium released with very little change in the time course of this release. As a result, the amplitude of the twitch increases with only small changes in time course. The slightly shortened time to peak force can be explained by the sarcoplasmic reticulum sequestering calcium more rapidly when the free calcium level is higher, such that relaxation begins slightly earlier.

Caffeine is known to impair calcium accumulation by the sarcoplasmic reticulum. Once calcium is released, its reaccumulation is delayed. The higher forces seen with caffeine can, therefore, be explained, at least in part, by the greater time for force development. The force-velocity curves must be examined to see how different changes in the isometric twitches could be accounted for the observed differences in isometric twitches. The force-velocity curves must be examined to see if it is necessary to postulate any additional changes in the contractile properties of the muscle.

Some experiments with skinned skeletal muscle fibers have shown that changes in activation do not alter maximum velocity but depress velocities at all finite loads. In addition, force-velocity curves obtained at different levels of activation can be made to superimpose if velocity is plotted against the relative rather than the absolute force, as was done in Figure 5B. It should be mentioned that this is still a somewhat controversial area (see review by Podolin and Ford), but over the range of conditions studied here (i.e., assessing just the first few milliseconds of shortening and at levels of activation between maximum and about half maximum), most recent studies indicate that maximum velocity does not change with activation. In the present study, the relative force-velocity curves obtained in different inotropic states do not superimpose exactly as would be

### Calcium Binding to Myofilaments

McClellan and Winegrad have developed a chemically skinned cardiac muscle preparation that can be activated and relaxed by changing the external calcium ion concentration in the presence of MgATP (see review by Winegrad). This preparation retains its membrane-bound hormone receptors so that the contractile response to calcium after hormonal intervention can be altered. β-Adrenergic stimulation decreases the preparation's sensitivity to calcium; that is, it requires a higher calcium ion concentration to achieve a given level of partial force. The decreased calcium sensitivity is associated with phosphorylation of troponin, the calcium-binding protein on the thin filaments. In this preparation, therefore, one effect of β-adrenergic stimulation is the lowering of the affinity constant for calcium binding to the myofilaments.
expected from the results obtained in skeletal muscle. In a previous study of the effects of changing activation on the force-velocity properties of cat papillary muscles, a similar result was obtained. Force-velocity curves were measured at different times in the twitch and, therefore, at different levels of activation. The relative force-velocity curves superimposed nearly, but not exactly. The differences in velocity at each load could either be attributed to a direct effect of activation on the contractile elements or to a small internal load.

**Internal Load**

In the presence of an internal load, the contractile elements cannot be fully unloaded, and the true maximum velocity cannot be achieved. Different maximum velocities are seen at different levels of activation because the internal load is a different fraction of the isometric force. Maximum velocity is affected more than other parameters used to describe the curve because the force-velocity curves are very steep at low loads and small changes in these low loads can cause large changes in the intercept of the curves with the velocity axis.

In an earlier study of cat myocardium at different times in the twitch, an internal load equivalent to 6% of the isometric force at the peak of an unpotentiated control twitch could have caused all of the differences in the relative force-velocity curves. Such a load would be doubled, to 12% of isometric force, when activation had risen to only half its peak value and could have reduced the apparent maximum velocity by 20%, as was found. In the present study, in which isometric force was increased twofold, an internal load equivalent to 4% of the isometric force would have been reduced to 2% of isometric force, and measured maximum velocity would have been increased by 5–10%, approximately what was found here. A constant internal load will cause the entire force-velocity curve to shift horizontally. Inspection of the curves in Figure 5C shows that a leftward shift of the potentiated curve by 2% of isometric force would cause the curves to almost coincide. The coincidence would not be exact, but the differences over most of the curve would be less than 1% of isometric force. The greatest difference would occur in the high-load low-velocity points. This observation suggests that, if there is an internal load, it may be shortening dependent; that is, it may be some type of viscoelasticity, as previously described. These considerations indicate that the changes in maximum velocity described here can be accounted for by a small internal load. They do not, however, prove that this is the cause of the changes seen. On the basis of the data presented, it is equally possible that the changes are due to some other effect of the potentiating agent on the force-velocity relation. The important point to be made about these experiments is that the effects of all of the interventions are the same. This observation suggests, but does not prove, that the changes produced by the different agents have the same effect on the contractile machinery.

**Changes in the Contractile Machinery**

Force and shortening in muscle is believed to be caused by the force-generating crossbridge that forms between the thick and thin myofilaments. These crossbridges, which are a structural part of the myosin thick filaments, cyclically attach to the actin thin filaments, generate force, and detach. In generating force, the attached bridges move through a limited range of motion and stretch a spring somewhere in the bridge. Changes in the amount of isometric force can be produced either by a change in the number of attached bridges or in the average force per attached bridge, that is, by a shift in the average position of an attached bridge. Measurements of isometric force alone cannot distinguish between these two mechanisms. Increases in activation are believed to increase the number of attached force-generating bridges by making more of the thin filament attachment sites receptive. Thus, increases in activation increase the number of attached force-generating bridges without influencing the average force per bridge. By contrast, an intervention that changes the average force per bridge would have to shift the average position of an attached bridge. Such a shift would be likely to influence the cycling kinetics of the bridges. Since shortening velocity is dependent on the cycling rate of the bridges, an inotropic agent that influences the average force per bridge would also be likely to change shortening velocity. This is the reason for our measuring the force-velocity relation as well as the isometric force response in the present study.

We chose postextrasystolic potentiation as one of the interventions because of the belief that its onset of action is so brief that there would be little time to produce a change in the chemistry of the contractile proteins. If this is so, then the effects of other agents could be compared with this intervention to see if they differed in some way. The principal finding in this study was that none of the chemical agents caused an effect that was different from postextrasystolic potentiation.

It is likely that some interventions might change the contractile machinery in a way that would be manifested as changes in the force-velocity properties. Pagani and Julian, for example, have shown that a change in the myosin type within the muscle causes profound changes in the force-velocity properties. Cardiac muscle can contain three types of myosin made up of two types of myosin heavy chains, α and β. Homodimers, either V$_1$ or V$_3$, are made of two α- or two β-myosin heavy chains and have fast or slow ATPase rates, respectively. A heterodimer, called V$_2$, is made of one α- and one β-myosin heavy chain and has an intermediate ATPase rate. The shortening velocity of the muscle is strongly dependent on the myosin type within the muscle. Muscle containing mostly V$_1$ myosin short-
ens about six times faster than muscle containing mostly V3 myosin.16

As mentioned above, McClellan and Winegrad and their colleagues25,26,36 have shown that β-adrenergic stimulation decreases the sensitivity of the myofilaments to calcium. They have further shown that β-adrenergic stimulation increases isometric force at supramaximal levels of activating calcium and that this increase occurs only in proportion to the relative amount of V3 myosin in the muscle (see review by Winegrad27). Hoh et al37 found that β-adrenergic stimulation of rat papillary muscle containing a predominance of V1 myosin increases the frequency of the minimum in dynamic stiffness plots, an effect that they interpret as being due to faster crossbridge cycling. These effects can be interpreted either as increases of a selective activation of the fast forms of myosin or of changes in the contractile kinetics of the fast form of myosin. Thus, it seems quite likely that β-adrenergic stimulation could, in some circumstances, increase shortening velocity to a much greater extent than postextrasystolic potentiation.

This inability to see a substantial change in shortening velocity with β-adrenergic stimulation in the present study may be due to the preponderance of V3 myosin in the adult euthyroid animals studied here.38 Because some types of inotropic interventions can cause a change in the proportion of the different types of myosin being activated, it is important that the muscles being studied have a preponderance of one type of myosin when changes in the dynamics of the crossbridge cycle are sought.

Comparison With Earlier Studies

The original studies of the influence of inotropic stimulation on the force-velocity properties of papillary muscle used afterloaded contractions2-4 and purported to show that maximum velocity was very strongly dependent on inotropic state. Similar conclusions were reached from studies in which maximum velocity was determined during a period of continuous shortening at very low loads.39 These types of studies have been criticized severely because the measurements were made at different times in the twitch, at different contractile element lengths, and after different amounts of shortening.5-7 The present study shows that when all the force-velocity points are made at the same time in the twitch and at the same contractile element length, the maximum velocity varies very little with inotropic state. This observation again suggests that conclusions drawn about inotropic state depend very strongly on the way the measurements are made.

References


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**KEY WORDS** • papillary muscle • heart muscle • inotropic interventions • acetylstrophanthidin • isoproterenol • caffeine
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