Caffeine Reversal of Length-Dependent Changes in Myocardial Contractile State in the Cat

LEONARD H.S. CHUCK AND WILLIAM W. PARMLEY

SUMMARY The present study evaluated potential mechanisms for the slow length-dependent change in myocardial contractile state. Using 40 isolated right ventricular cat papillary muscles, we found that 10 mM caffeine reversed the subsequent slow change in myocardial performance following a change in muscle length. Since caffeine acts both at the sarcolemma and the sarcoplasmic reticulum, we attenuated the sarcolemmal influx of calcium with verapamil, manganese, and low external calcium concentration. None of these interventions altered the caffeine reversal of the length-dependent effect. It thus appears that the length-dependent alteration of contractile state is of intracellular origin, and probably related to altered calcium handling by the sarcoplasmic reticulum.


THE relationship between muscle length and cardiac performance has been described by the Frank-Starling relationship. After an increase in end-diastolic fiber length along the ascending limb of this relationship, there is an increase in cardiac performance. At any given length, positive inotropic agents can further increase cardiac performance. Thus, the traditional view was that these two mechanisms for altering cardiac performance were independent of each other. The Frank-Starling relationship was explained by a variation in the number of cross-bridges formed between the contractile proteins (Gordon et al., 1966). The positive inotropic response was explained by an increase in the rate of interaction of these cross-bridges (Katz, 1970).

Contrary to the traditional belief that these two mechanisms for altering cardiac performance were separate from one another, we reported a slow length-dependent change in myocardial contractile state (Parmley and Chuck, 1973). In isolated cat papillary muscles, the immediate response to an increase of muscle length was followed by a slow additional increase in performance. Thus, in addition to the instantaneous length, the length history becomes an important determinant of the “Frank-Starling” response. Subsequent studies have suggested that the immediate increase in force along the ascending limb is due not so much to myofilament and cross-bridge overlap, but to a length-dependent alteration in the amount of calcium activating these cross-bridges (Allen et al., 1974; Jewell, 1977; Lakatta and Jewell, 1977; Fabiato and Fabiato, 1975). This recent evidence further questions the traditional separation between alterations in performance due to length and contractility.

In our early attempts to identify a mechanism for the slow time-dependent coupling between muscle length and contractile state, we ruled out cross-sectional area, release of stored catecholamines, temperature effects (24°-37°C), or series viscous elements as mechanisms for this observation (Parmley and Chuck, 1973). We did find, however, that varying the external calcium concentration changed the magnitude of the slow response, suggesting a calcium-mediated mechanism. In this paper, we report additional studies designed to investigate the effect of altered intracellular calcium on this phenomenon.

Methods

Forty right ventricular papillary muscles were rapidly obtained by a left thoracotomy from cats anesthetized with sodium pentobarbital (40 mg/kg, ip). Each muscle was suspended vertically in a temperature-controlled (30°C) bath of Krebs-Henseleit solution, which was composed of (in mM) NaCl, 118.5; KCl, 4.74; MgSO$_4$, 2.43; KH$_2$PO$_4$, 1.18; NaHCO$_3$, 24.9; CaCl$_2$ • 2H$_2$O, 2.54; and dextrose, 5.0. The bath was gassed with a mixture of 95% O$_2$ and 5% CO$_2$ and was maintained at a pH of 7.4. The muscles were stimulated 10% above threshold by an AEL 104A stimulator attached to punctate platinum electrodes. These lightly preloaded (<0.4 g/mm$^2$) muscles were first allowed to stabilize by contracting isometrically every 5 seconds for at least 1 hour. L$_{max}$ (muscle length at which maximal force was developed) was then determined by slowly lengthening each muscle while recording resting force, developed force, and dF/dt$_{max}$. As shown in Table 1, only muscles with resting tension-to-total tension ratios of less than 0.15 in normal calcium or 0.20 in 1.27 mM calcium-Krebs solution were studied (Brutsaert and Claes, 1974).
Length dependence of contractile state was studied in the following way: Muscle length was shortened by 10% from L_{max} and both the immediate decrease and the slow additional decrease in isometric force and rate of force development were monitored at slow speed on a Brush Mark 200 ink recorder. Force and dF/dt were used as indicators of cardiac performance in these isometrically beating muscles. Selected fast-sweep Polaroid records were taken off a Tektronix 5103 storage oscilloscope after the immediate decrease in performance and again after performance was stable. In a similar fashion, performance was monitored after an abrupt lengthening almost to L_{max}.

We next determined the effects of caffeine on this slow length-dependent change of myocardial contractile state. Caffeine was added from a stock solution of 100 mM caffeine in Krebs solution. Final bath concentrations were 1, 5 and 10 mM caffeine. We waited 40 minutes before studying the slow changes of performance caused by a length change. Performance was monitored during these 40 minutes to establish that each muscle stabilized following its biphasic mechanical response to caffeine (Shine and Langer, 1971). During exposure to caffeine, the length dependence of contractile state was studied by shortening and then lengthening each muscle, as outlined above.

Because caffeine reversed the length-dependent phenomenon, we attempted to modify the caffeine effect by altering the sarcolemmal calcium flux in three ways:

1. Verapamil (Isoptin, Knoll Pharmaceutical) was added from a 10^{-3} M stock solution of this drug in Krebs solution. Cumulative dose-effect curves were constructed to identify the verapamil dose which still allowed adequate measurement of the length dependence. The effects of 10^{-6} and 10^{-5} M verapamil alone on the length-dependence of contractile state were studied 40 minutes after its addition when stable performance had been reestablished. The combined effects of verapamil and caffeine on the length-dependence of contractile state were studied 40 minutes after the addition of caffeine.

2. Manganese (3 mM) was added from a 0.3 M stock solution in a manner similar to that of Langer et al. (1975). Caffeine was then added from a 100 mM stock solution as indicated above.

3. The length-dependence then was studied in 1.27 mM calcium (1/2 normal). These experiments were begun with the muscles in this low calcium concentration. Caffeine was dissolved in low calcium Krebs solution and added to the bath to achieve a final concentration of 10 mM. Forty minutes later, the ability of caffeine to reverse the length dependence was studied.

The effects of theophylline, a more specific phosphodiesterase inhibitor (Goodman and Gilman, 1970, Blinks et al., 1972) were tested in three muscles 40 minutes after addition of this drug to the bath. The final bathing concentration of theophylline was 10 mM.

Muscle length and weight were measured at the end of the experiment. Muscle cross-sectional area was calculated by assuming that the muscle was a cylinder with a specific gravity of 1.0. Mechanical performance was measured by monitoring developed force (F), maximum rate of force development (dF/dt), time-to-peak force (TTP), and the half-time for relaxation (RT_{1/2}) off fast-sweep records. The data were analyzed statistically by using a two-way analysis of variance and a Student-Newman-Keuls multiple range test on a mixed effects model.

Results

The primary observation of this study was that 10 mM caffeine reversed the direction of the length-dependent changes of myocardial contractile state as monitored by directional changes of both maximum dF/dt and force. Depicted in Figure 1 are slow-speed isometric force tracings of a typical muscle following length changes. All of the muscles studied in 10 mM caffeine showed these responses. At the top is the previously described secondary change in force following a length change. As the muscle is shortened from L_{max} to 90% of L_{max} (L_{90}), there is an initial (i) reduction in resting and developed force. Over the next 15 minutes there is a further reduction in developed force to a stable (s) level. This phenomenon is reversible since a subsequent lengthening to L_{max} produces an initial increase in resting and developed force (L_{max}), which is followed by a subsequent increase in force over 15 minutes to a stable level (L_{max}).

In the middle panel, the reversal of this length-dependent effect by 10 mM caffeine is illustrated. Following a reduction in muscle length to L_{90}, the initial reduction in resting force is followed by a slow increase to a stable level (L_{90}). A subsequent increase in muscle length to L_{max} produced an increase in force which was followed by a slow sub-

<table>
<thead>
<tr>
<th>Krebs Solution</th>
<th>Calcium (mM)</th>
<th>n</th>
<th>Length (mm)</th>
<th>Cross-sectional area (mm²)</th>
<th>Resting stress (g/mm²)</th>
<th>Total stress (g/mm²)</th>
<th>RF/TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Ca^{2+}</td>
<td>2.54 mM</td>
<td>32</td>
<td>7.4 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.04</td>
<td>6.2 ± 0.4</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Low Ca^{2+}</td>
<td>1.27 mM</td>
<td>8</td>
<td>7.0 ± 0.5</td>
<td>1.5 ± 0.3</td>
<td>0.6 ± 0.05</td>
<td>4.2 ± 0.4</td>
<td>0.14 ± 0.02</td>
</tr>
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</table>
Caffeine (10 mM) reversed the length-dependent changes of myocardial contractile state. In normal Krebs solution (top panel) when this representative muscle was shortened from \( L_{\text{max}} \) to 90% of \( L_{\text{max}} \) (\( L_{90} \)) force development decreased immediately and continued to decrease with time until it stabilized at \( L_{90} \). Lengthening the muscle back to \( L_{\text{max}} \) (\( L_{\text{max}} \)) caused both an immediate increase of force and a more gradual increase of force to \( L_{\text{max}} \). Caffeine (10 mM) reversed the direction of this length dependence (middle panel). Shortening the muscle caused a gradual increase of force, and lengthening caused a subsequent gradual decrease of force. Washing with normal Krebs solution restored the original pattern (lower panel). Muscle characteristics: \( L_{\text{max}} = 6.0 \) mm; cross-sectional area = 0.83 mm\(^2\), resting tension/total tension = 0.065; developed stress = 8.64 g/mm\(^2\).

Following removal of caffeine from the bath by washing, the original length-dependent changes were reestablished (bottom panel).

Although 10 mM caffeine increased the resting force after lengthening to \( L_{\text{max}} \) (middle panel of Fig. 1) compared to the decreased resting force under normal and post-wash conditions, this was not a consistent finding. This resting force change both increased and decreased after 10 mM caffeine and a length change but only amounted to 0.03 ± 0.10 g on average after lengthening to \( L_{\text{max}} \). Using the halftime of relaxation (RT\(_{1/2}\)) as a crude measure of relaxation, there were 14.4 ± 0.7 RT\(_{1/2}\) interposed between contractions.

Only at a bathing concentration of 10 mM was caffeine able to reverse the direction of the length-dependent changes. At concentrations of caffeine below 10 mM, the directional changes were similar to those of the initial control and the final control after washing. Depicted in Figure 2 are the average changes in isometric stress (g/mm\(^2\)) from all of the muscles studied at different doses of caffeine, at \( L_{90} \) and \( L_{\text{max}} \) as in Figure 1, and at the times indicated in Figure 1. There was no directional alteration in the control length-dependent response following the addition of 1 or 5 mM caffeine. The altered directional response to 10 mM caffeine, however, is clearly seen.

Caffeine (10 mM) produced slight changes in the time-to-peak force during the slow time-dependent changes of performance (Fig. 3). Illustrated in panel A of Figure 3 are the isometric force traces obtained from the same muscle and during the protocol illustrated in Figure 1. As noted above, at this dose, all the muscles studied responded in this manner. The bottom two traces show the initial (i) and stable (s) contractions following a reduction in muscle length from \( L_{\text{max}} \) to \( L_{90} \). The top two tracings show the initial and stable contractions following an increase in muscle length back to \( L_{\text{max}} \). Although time-to-peak force is longer at \( L_{\text{max}} \) than at \( L_{90} \), there is no significant difference in this measurement between the initial and stable contractions at each length. The comparable tracings in 10 mM

**Figure 1** Caffeine (10 mM) reversed the length-dependent changes of myocardial contractile state. In normal Krebs solution (top panel) when this representative muscle was shortened from \( L_{\text{max}} \) to 90% of \( L_{\text{max}} \) (\( L_{90} \)) force development decreased immediately and continued to decrease with time until it stabilized at \( L_{90} \). Lengthening the muscle back to \( L_{\text{max}} \) (\( L_{\text{max}} \)) caused both an immediate increase of force and a more gradual increase of force to \( L_{\text{max}} \). Caffeine (10 mM) reversed the direction of this length dependence (middle panel). Shortening the muscle caused a gradual increase of force, and lengthening caused a subsequent gradual decrease of force. Washing with normal Krebs solution restored the original pattern (lower panel). Muscle characteristics: \( L_{\text{max}} = 6.0 \) mm; cross-sectional area = 0.83 mm\(^2\), resting tension/total tension = 0.065; developed stress = 8.64 g/mm\(^2\).

**Figure 2** Concentration-dependent effects of caffeine on the length dependence of myocardial contractile state. Stress development (mean ± SE) from groups of muscles studied at different doses of caffeine and at \( L_{\text{max}} \) or \( L_{90} \) (90% of \( L_{\text{max}} \)). The directional response in normal Krebs solution (\( \bigcirc, n = 15 \)) was not changed by the addition of 1 mM caffeine (\( \bigtriangleup, n = 10 \)) or 5 mM caffeine (\( \triangle, n = 6 \)). This was also true after washing with normal Krebs solution (\( \blacklozenge, n = 11 \)). Caffeine (10 mM) however, (\( \bigodot, n = 10 \)) reversed the direction of this response.
Figure 3 Fast-sweep oscilloscopic records of initial (I) and stable (S) force traces after changing the length of the same muscle as shown in Figure 1. In normal Krebs solution (panel A), there was no change of the time-to-peak force (TTP) at either the shorter length (lower two tracings) or the longer length (upper two tracings). In 10 mM caffeine, the direction of the force development between the initial state (I) and the stable state (S) was reversed (panel B). Caffeine (10 mM) also changed the time-to-peak force (TTP) associated with this slow change in force development at each muscle length. After washing with normal Krebs solution, the original response returned (panel C).

Figure 4 Concentration-dependent effects of caffeine on the slow changes of time-to-peak force between L90% and L90, and between Lmax and Lmax. The averaged changes of time-to-peak are plotted as a percentage of the control time-to-peak under two conditions: at the shorter length (L90) and at the longer length (Lmax). The data represents the mean ± SE for 19 muscles at three concentrations of caffeine (shaded area). The asterisk indicates data that is statistically different from the control at a level of \( p < 0.01 \).

Discussion

The fact that caffeine reverses the length-dependent changes in contractile state (Fig. 1) suggests that it must affect the mechanism responsible for this phenomenon. If the primary mechanism for this length dependence was an increase in transsarcolemmal calcium flux, inhibition of this calcium...
It appears, therefore, that the length-dependent changes of myocardial contractile state are mediated by an intracellular mechanism. Because our measurements of force development only indirectly monitor the activation levels of intracellular calcium, we can only infer the intracellular mechanism by which caffeine directionally alters the length dependence of contractile state. Caffeine has four reported intracellular actions which might be responsible. (1) Caffeine rapidly releases calcium from the sarcoplasmic reticulum (SR) in both skeletal and cardiac muscle (Weber and Herz, 1968; Endo et al., 1970; Fabiato and Fabiato, 1975). (2) Caffeine decreases the rate of calcium uptake by the SR (Weber, 1968; Shine and Langer, 1971). This decreased uptake would account for the prolonged relaxation (Blinks et al., 1972; Henderson et al., 1974) which might give rise to incomplete relaxation and hence an increase of resting force. In these experiments at 10 mM caffeine, however, the 14+ half-times for relaxation would have provided sufficient time for complete relaxation. No consistent increase of resting force was found. (3) Caffeine increases the calcium permeability of the sarcolemma (Nayler, 1963; Blinks et al., 1972; Kavalier et al., 1978) and vesicles (Blayney et al., 1978). This increased permeability and potentially increased calcium efflux from the SR depends on the relative internal and external calcium concentrations (Katz...
et al., 1977). (4) As a phosphodiesterase inhibitor, caffeine increases intracellular levels of cyclic AMP (Butcher and Sutherland, 1962). This increase of cyclic AMP might lead to protein-kinase-catalyzed phosphorylation of phospholamban and an increased calcium uptake by the SR calcium pump (Hicks et al., 1979; Tada et al., 1979). Caffeine, however, only increases the myofibrillar ATPase activity at a concentration of 20 mM (Blayney et al., 1978).

Our current finding that theophylline, a more potent phosphodiesterase inhibitor (Goodman and Gilman, 1970), produces exactly the same reversal as does caffeine would indicate that this phosphodiesterase inhibition may not be the primary controlling factor. Caffeine and theophylline have similar actions on mechanical activity (Blinks et al., 1972) so this similarity of action is not unexpected.

Some combination of these different effects of caffeine might alter the observed length-dependent changes of myocardial contractile state. Changes in muscle length could give rise to (1) altered calcium release from intracellular stores, and/or (2) altered calcium uptake into intracellular stores. Potential explanations for the length-dependent phenomena follow.

If an increase in muscle length caused a time-dependent increase in the calcium released from intracellular sites, then we would expect a gradual increase in contractile state. Frank and Winegrad (1976) have shown decreased calcium efflux from skeletal muscles after lengthening these muscles. They observed morphological changes of the terminal cisternae suggesting a mechanism for the alteration of intracellular calcium release. Katz et al. (1977) found that the calcium permeability of SR vesicles from rabbit skeletal muscle depended on the intravesicular and extravesicular calcium concentrations. Similarly, Dunnett and Nayler (1978) have shown an increased net calcium efflux from cardiac SR vesicles with an increase in extravesicular calcium. Other studies in which the free intracellular calcium was monitored by using the calcium sensitive dye, aequorin, showed either a decreased free calcium in stretched skeletal muscles (Blinks et al., 1978) and stretched atrial muscles (Allen and Blinks, 1978) or no change in free calcium in stretched Purkinje fibers (Wier, 1979).

If an increased muscle length altered both SR calcium release and uptake, then the gradual increase in contractile state could be explained by a predominance of calcium release. Caffeine, however, markedly increases calcium release and decreases the stores of calcium by increasing the calcium permeability of the SR (Blayney et al., 1978). The mechanical effects of caffeine are a delayed onset of activation (Henderson et al., 1973), increased time-to-peak force and slowed relaxation (Henderson et al., 1974). In the presence of these major effects of caffeine, lengthening the muscle might not further increase SR calcium release. The increased muscle length, however, might still increase uptake and thereby reverse the direction of the length-dependent changes. Such a speculative mechanism would be consistent with the observed changes induced by length and caffeine.

The slow length-dependent phenomena might reflect much more complex alterations of intracellular calcium handling. Until these intracellular mechanisms are fully tested in appropriate models, the precise cause of this length dependence and its reversal by caffeine will remain a matter of speculation.

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L H Chuck and W W Parmley

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