β-Adrenergic Receptor Stimulation Increases Unloaded Shortening Velocity of Skinned Single Ventricular Myocytes From Rats

Kevin T. Strang, Nancy K. Sweitzer, Marion L. Greaser, Richard L. Moss

Abstract In vitro biochemical experiments have suggested that stimulation of β-adrenergic receptors may increase the rate of crossbridge cycling in mammalian myocardium, but recent attempts to demonstrate a mechanical correlate have yielded conflicting results. To investigate this issue, we measured the effect of isoproterenol (ISO) and cAMP-dependent protein kinase (PKA) on unloaded shortening velocity (Vₜ). Vₜ is thought to be determined by the rate-limiting step of the crossbridge cycle, i.e., the rate of crossbridge detachment from actin, and is therefore an index of the cycling rate. Single rat ventricular myocytes were enzymatically isolated, incubated in Ringer’s solution without (control) or with 0.1 μmol/L ISO, and then rapidly skinned. Some control cells were subsequently treated with 3 μg/mL PKA for 40 minutes. Vₜ was then measured during maximal activation (pCa 4.5) in control, ISO-treated, and PKA-treated cells using the slack-test method. To test the efficacy of the agonist treatments, Ca²⁺ sensitivity of isometric tension was also assessed for each treatment by determining the [Ca²⁺] required for half-maximal tension (ie, pCa⁰). Both ISO and PKA treatment reduced the Ca²⁺ sensitivity of isometric tension compared with same-day control cells, in agreement with previous studies in intact and in skinned preparations. Vₜ was increased 38% by ISO treatment and 41% by PKA treatment compared with same-day control cells. ³²P autoradiography showed that troponin I and C protein were the principal proteins phosphorylated by PKA treatment. We conclude that β-adrenergic stimulation increases the rate of crossbridge release from actin, by a mechanism that most likely involves the phosphorylation of troponin I and/or C protein by PKA. (Circ Res. 1994;74:542-549.)

Key Words β- adrenergic receptors • cardiac myocytes • shortening velocity • phosphorylation

Stimulation of β-adrenergic receptors has been proposed to increase the rate of crossbridge cycling in mammalian myocardium. If true, this may contribute to increases in twitch tension and/or the rates of force development and relaxation observed during adrenergic stimulation of cardiac muscle. In vitro experiments showed an increase in Ca²⁺-activated actomyosin ATPase activity in response to the β-receptor agonist isoproterenol (ISO), suggesting that the cycling rate is increased, but attempts to demonstrate a mechanical correlate have yielded conflicting results. ISO-induced increases in the frequency of minimum stiffness (fₘₙᵣₚ) of papillary muscles in barium contracture have been interpreted to reflect an increase in crossbridge cycling rate. On the other hand, other studies on intact papillary muscles found no β agonist–induced changes in the velocity of unloaded shortening (Vₜ), either when measured by extrapolation of the force-velocity relation or by use of an isovelocity release technique during a twitch. Vₜ is thought to be determined by the rate of detachment of myosin heads from actin, and biochemical experiments suggest that ADP dissociation from myosin is sufficiently slow to be the rate-limiting step in crossbridge dissociation. The absence of an effect on Vₜ therefore implies that the crossbridge cycling rate is not changed by β-adrenergic stimulation. Vₜ and fₘᵣₑ results are not necessarily contradictory, however, because these mechanical properties characterize crossbridge cycling under different conditions. The increase in fₘᵣₑ was observed during isometric contraction in which crossbridges are under considerable strain. On the other hand, shortening under low or zero load is characterized by less strained, and perhaps even compressively strained, crossbridges. Conceivably, β-adrenergic stimulation may modulate the strain dependence of one or more step(s) in the crossbridge cycle, which might account for different effects under the two conditions.

Alternatively, the previous experiments may have led to different conclusions because of the difficulties associated with interpreting experiments on intact multicellular preparations. First, the profound alteration in the dynamics of Ca²⁺ handling during β-adrenergic stimulation of living myocardium makes it difficult to resolve fundamental alterations in the crossbridge cycling rate because Vₜ varies with [Ca²⁺]. Also, multicellular preparations such as papillary muscles and trabeculae generally have compliant attachments to the mechanical apparatus and invariably have substantial connective tissue in parallel with the myofilaments. These problems make it difficult to infer crossbridge function on the basis of mechanical measurements in multicellular preparations because of uncertainty concerning the relative contributions of active and passive viscoelastic elements to mechanical properties.

To circumvent these problems, we investigated the effect of β-adrenergic receptor stimulation on Vₜ in single skinned cardiac myocytes from rats. Vₜ was measured by the slack-test method and was compared between control and ISO-treated cells from the same
whole-heart preparation, which were rapidly skinned to preserve the myofibrillar phosphorylation state. In another series of experiments, \( V_o \) was compared between untreated control cells and control cells incubated with the catalytic subunit of cAMP-dependent protein kinase (PKA), and myofilament protein phosphorylation was assessed under those same conditions using \( ^{32} \)P autoradiography. Ca\(^{2+}\) sensitivity of isometric tension was also measured in both ISO- and PKA-treated cells to assess the response of cells to these agonists. Both ISO and PKA treatment significantly reduced Ca\(^{2+}\) sensitivity of tension in agreement with earlier results, and in addition, both treatments increased \( V_o \). \( ^{32} \)P autoradiography suggests that phosphorylation of troponin I (TnI) and/or C protein may be responsible for these effects.

### Materials and Methods

Single ventricular myocytes were obtained using a modification of the technique described by Haworth et al. Female Sprague-Dawley rats were anesthetized with methoxyflurane, and the heart was rapidly excised. The aorta was cannulated with a short segment of polyethylene tubing, which was then inserted into a modified Langendorff apparatus for retrograde perfusion via the coronary circulation. The heart was initially perfused with 75 mL of Ringer’s solution containing (mmol/L) MgCl₂ 1.2, CaCl₂ 1, KCl 4.8, NaCl 118, KH₂PO₄ 2, pyruvate 5, glucose 11, insulin 1, and HEPES 25 (pH 7.4), followed by a 4.5-minute perfusion with Ca\(^{2+}\)-free Ringer’s solution. Next, Ringer’s solution containing 1.0 mg/mL collagenase (class I, Worthington), 0.4 mg/mL hyaluronidase, 20 mmol/L taurine, and 0.05 mmol/L CaCl₂ was perfused for 11 minutes, after which the heart was minced and incubated with gentle shaking for 20 minutes in 10 mL of the same solution plus 0.25% bovine serum albumin. After filtering through 300-μm Teflon mesh to remove undigested materials, the cells were washed and resuspended in Ringer’s solution with 1 mmol/L Ca\(^{2+}\). All solutions were 37°C and bubbled with 100% O₂. Chemicals were purchased from Sigma, unless otherwise indicated.

Cells were divided into aliquots, resuspended in 1 mmol/L Ca\(^{2+}\)-Ringer’s solution with or without 0.1 μmol/L ISO for 2 minutes at 37°C, and then rapidly skinned by resuspension for 6 minutes at 22°C in a relaxing solution containing (mmol/L) free Mg\(^{2+}\) 1, KCl 100, EGTA 2, ATP 4, and imidazole 10 (pH 7.0) plus 0.3% Triton X-100 (Ficoll). This procedure preserves myofibrillar proteins in a given phosphorylation state by quickly removing soluble and membrane-bound kinases and phosphatases. The cells were then washed twice in fresh relaxing solution and stored on ice until use. To investigate the effects of PKA, some control skinned cells obtained by the above procedure were incubated at 20°C for 40 minutes in relaxing solution containing the catalytic subunit of porcine cardiac PKA (3.0 μg/mL) immediately before use. All relaxing and activating solutions had an ionic strength of 180 mmol/L.

Single cells were attached with silicone adhesive (Dow Corning) to glass micropipettes on the stage of a Zeiss inverted microscope modified for temperature control, as previously described (see Fig 1). One pipette was fixed to a piezoelectric translator (Physik Instrument Co, Waldbronn, Germany) and the other to a force transducer (model 403, which has a sensitivity of 20 mV/mg and resonant frequency of ~300 Hz; Cambridge Technology, Cambridge, Mass), both of which were mounted on micromanipulators (Narishige). The output signal from the force transducer was amplified 10-fold; then the signal was input into an oscilloscope (model NIC-310, Nicolet Instrument Corp, Madison, Wis) for storage on magnetic disk and subsequent analysis. The piezoelectric device was driven by a bipolar operational power supply/amplifier (Kepco Inc, Flushing, NY), which is linear to ±50 μm at a calibration of 0.054 μm/V. The amplifier output signal was monitored on a second channel of the oscilloscope. The amplifier was driven by voltage-command signals from a pulse-interval generator (World Precision Instruments Inc, New Haven, Conn). Sarcomere length (SL) and cell width were monitored and recorded either by means of photomicrographs taken with a 35-mm camera back (Nikon) or by videotape (Panasonic video camera model WV-B1600) and a VHS recorder (JVC HR-s6000u). Cell depth was measured in some cells by detaching one pipette and rotating the cell 90° with the remaining pipette. On average, the depth was 91 ±9% of the width (n = 19). Cross-sectional area was calculated for all cells on the basis of an elliptical shape; in cells for which only width measurements were available, the depth was assumed to be 91% of the width. Ca\(^{2+}\) sensitivity of isometric tension was determined under control conditions and after ISO and PKA treatment as follows: Isometric tension was measured during
maximal activation (pCa 4.5) at the beginning, middle, and end of each experiment to assess the performance of the preparation; data were discarded if maximum tension declined >20%. In between, tension was measured at varying submaximal pCa values and expressed as a fraction of the maximum tension (P_{n0}). The data were analyzed by least-squares regression using the Hill equation:

\[
\log\left[\frac{P_{n0}}{1-P_{n0}}\right] = n \log[Ca^{2+}] + k
\]

where \( n \) is the Hill coefficient, and \( k \) is the intercept of the fitted line with the \( x \) axis, which corresponds to the \([Ca^{2+}]\) at half-maximal isometric tension (pCa_{0.5}). By use of the constants derived from the Hill equation, tension-pCa curves were fitted by computer with the following equation:

\[
P_{n0} = \frac{[Ca^{2+}]^n}{[Ca^{2+}]^n + [Ca^{2+}]^k}
\]

SL was initially set at 2.30 μm and was monitored during activation. Cells were considered too compliant and were thus discarded if SL varied by >0.2 μm between relaxed and maximally activated conditions. The experimental chamber was cooled to 15°C by use of thermoelectric devices (Cambion, Midland-Ross Co, Cambridge, Mass), which in turn were cooled by circulating water.

For the determination of unloaded shortening velocity, as illustrated in Fig 2. Once steady tension was achieved, cells were slackened by varying amounts from an initial SL of 2.3 μm, and the time required to take up the slack was measured from the beginning of the length step to the onset of tension redevelopment. The maximum step size imposed was such that cells were not allowed to shorten below an SL of 1.8 μm, at which point interference from restoring forces is likely to occur.12,20 The time point that tension redevelopment was determined by hand fitting a line through the tension baseline and determining its intersection with a line drawn through the initial portion of the tension rise. Relative length change was plotted versus the duration of unloaded shortening. The scope of this plot was determined by linear regression and was recorded as V<sub>c</sub>.13 Ca<sup>2+</sup> sensitivity of isometric tension was assessed in each cell, either by characterizing a complete tension-pCa relation after the slack test or by measuring tension at a single submaximal \([Ca^{2+}]\) (pCa 5.7). Criteria for discarding cells because of excessive compliance or loss of tension were as above. Additionally, data from a given cell were not used if fewer than four data points were obtained or if the \( r \) value was <.95 for the fitted lines.

To account for differences in V<sub>c</sub> that could arise from myosin isoform differences between cells,21 the relative proportions of fast and slow myosin heavy chain (MHC) were determined for each individual cell using sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) as previously described.20 To account for variations between individual rat heart preparations, the pCa_{0.5} and V<sub>c</sub> from same-day control and agonist-treated cells were paired and analyzed using Student’s paired t test. Differences were deemed significant at \( P<.05. \) pCa_{0.5} and V<sub>c</sub> were in some cases both determined in the same cell, and on some days the same control cell was paired with both ISO- and PKA-treated cells. In parallel experiments, aliquots of skinned myocyte suspensions were incubated with PKA under the same conditions described above, with the addition of \[^{32}P\]y-ATP (Dupont) at a specific activity of 25 μCi/μmol. The reaction was terminated after 10, 20, or 40 minutes by the addition of electrophoresis sample buffer (8 mol/L urea, 2 mol/L thiourea, 75 mmol/L dithiothreitol, 3% SDS, 1% bromophenol blue, and 50 mmol/L Tris, pH 6.8) and heating at 100°C for 3 minutes. Sample volumes of 10 μL were subjected to SDS-PAGE.22 Gels were subsequently stained with Coomassie blue, dried, and exposed to x-ray film (X-OMAT AR, Eastman Kodak Co) for 10 days.

---

**Fig 2.** Method of determining unloaded shortening velocity. A. Expanded force vs time recordings were obtained during three different-size slack steps (16.6%, 18.5%, and 20.4% of muscle length) in a control cell. The duration of unloaded shortening was determined as the time from the beginning of the imposed length step to the beginning of tension redevelopment. The time of tension redevelopment was determined as the intersection of lines fit by eye through the zero-force baseline and the initial portion of tension redevelopment. Inset: Unexpanded tracings of length steps (upper) and force vs time (lower) for the same three slack steps. Average total force, measured as the drop in tension after the slack step, was 1.25 mg. B. Slack-test plot shows the data for all slack steps in this cell: unloaded shortening velocity was 1.71 muscle lengths (ML)/s.

**Results**

The attachment procedure consistently provided low-compliance attachments of single myocytes in which sarcomeres could be monitored both at rest and during activation (Fig 1). SL shortened an average of 0.07 μm (ie, ~3%) when myocytes were maximally activated compared with the relaxed condition. Mean dimensions and force characteristics for all cells studied (\( n=53 \)) were as follows: cell length, 97.3±20 μm; cross-sectional area, 364±203 μm²; maximum isotonic tension, 36.6±15 kN/m²; and resting tension, 2.85±1.3 kN/m². The proportion of the different MHC isoforms did not vary
significantly between treatment groups: average MHC composition was 81% α-MHC and 19% β-MHC for all cells.

ISO treatment caused a rightward shift in the isometric tension-pCa relation compared with the control condition in every pair of cells studied (Fig 3 and Table 1), indicating a reduction in the Ca²⁺ sensitivity of tension. There was no change in the apparent molecular cooperativity of activation as inferred from the slopes. Treating previously skinned control cells with the catalytic subunit of PKA had a similar effect on Ca²⁺ sensitivity as did β-receptor stimulation followed by rapid skinnning (Fig 4 and Table 1). The mean pCa₅₀ for the PKA group was higher than for the ISO group, in part as a result of variation in control values during some of the PKA experiments. However, the mean rightward shift in pCa₅₀ from same-day paired control cells (ΔpCa₅₀) was not significantly different in PKA-treated versus ISO-treated cells.

Both the ISO and PKA treatments significantly increased Vₚ compared with same-day control cells, and by similar amounts (Table 2). Fig 5 shows original tracings and slack-test plots from control, ISO-treated, and PKA-treated cells from a single whole-heart preparation. The original tracings show that, for a given change in slack-step size, there was a smaller increment in the time to tension redevelopment in the ISO- and PKA-treated cells than in the control cells, which resulted in a steeper slope in the slack-test plot. These three cells were also typical of the slack-test experiments in that the agonist-treated cells had lower y intercepts (the mean intercept for ISO cells was 2.8% of muscle length less than the intercept for control cells; the mean intercept for PKA cells was 5.3% of muscle length less than the intercept for control cells) and were less sensitive to Ca²⁺ compared with control cells (Fig 5b, inset; and Table 2). Clafin et al²³ have shown in skeletal muscle that the y intercept is in part determined by the extent of rapid initial shortening, which varies as a function of resting tension, but the slope of the slack plot (ie, Vₚ) was not affected by the level of initial resting tension. We investigated this idea by measuring resting tension in control and PKA-treated cells for which both width and depth measurements were available. Mean resting tension in a solution of pCa 9 and at an SL of 2.3 μm was 4.37±2.32 kN/m² in control cells and 2.45±0.79 kN/m² in PKA-treated cells (P<.02 by Student’s t test, n=11 for each group). Thus, it appears that PKA treatment reduces passive tension in cardiac myocytes, and on the basis of work by Clafin et al²³ in skeletal muscle, such a reduction would be expected to reduce the intercept of the slack-test plots in both β-agonist-treated and PKA-treated myocytes but would not be expected to influence Vₚ.

To directly investigate the possibility that β-adrenergic stimulation reduces resting tension, we measured resting tension in control cells before and after treatment with PKA (Fig 6). Resting tension was determined at 5-minute intervals by applying a 20% slack step from an initial SL of 2.3 μm. Resting tension began to decrease as soon as PKA was applied and reached a steady 56% of the initial value (filled circles in Fig 6). In another cell, no change in resting tension was observed in response to treatment with a previously boiled PKA solution, but resting tension dropped to 75% of control on exposure to native PKA (open circles in Fig 6). From these results, it is evident that treatment with β-agonist or PKA specifically reduces resting tension, most likely as a result of phosphorylation of myofilamentary proteins.

There was also a trend toward lower maximum isometric force per cross-sectional area in the agonist-
TABLE 2. Increase in Unloaded Shortening Velocity Compared With Same-Day Control Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$V_o$, ML/s</th>
<th>$P_{rel}$</th>
<th>$\Delta V$, %</th>
<th>$\Delta P_{rel}$, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.25±0.46</td>
<td>0.51±0.12</td>
<td>...</td>
<td>...</td>
<td>12</td>
</tr>
<tr>
<td>ISO</td>
<td>2.92±0.61</td>
<td>0.15±0.09</td>
<td>+38±30*</td>
<td>−36±12*</td>
<td>12</td>
</tr>
<tr>
<td>PKA</td>
<td>3.28±0.25</td>
<td>0.26±0.08</td>
<td>+41±15*</td>
<td>−31±10*</td>
<td>6</td>
</tr>
</tbody>
</table>

$V_o$ indicates unloaded shortening velocity; ML, muscle lengths; $P_{rel}$, fraction of maximal tension measured at pCa 5.7; ISO, isoproterenol; and PKA, cAMP-dependent protein kinase. Values are mean±SD; control values were pooled from all experiments.

*P<.001 by paired t test.

ISO, the force was 38.6±16.7 kN/m² in control cells and 27.8±12 kN/m² in ISO-treated cells; in experiments with PKA, the force was 50.6±9.1 kN/m² in control cells and treated groups than in paired control groups, but this result was not statistically significant because of substantial within-group variability. In experiments using

**FIG 5.** Unloaded shortening velocity in control, isoproterenol (ISO)-treated, and cAMP-dependent protein kinase (PKA)-treated cells. A, Original force vs time recordings from control (left column), ISO-treated (center column), and PKA-treated (right column) cells, as described in the legend of Fig 2. Cell lengths were 93, 105, and 132 μm in control, ISO-treated, and PKA-treated cells, respectively. Slack steps (percentage of muscle length) and duration of unloaded shortening (time in milliseconds) were as follows: for control cells, change in muscle length was 21.5%, 20.3%, and 19.1% for respective time values of 41.2, 37.0, and 29.0 milliseconds; for ISO-treated cells, change in muscle length was 21.6%, 20.6%, and 19.5% for respective time values of 42.2, 39.8, and 35.6 milliseconds; and for PKA-treated cells, change in muscle length was 20.2%, 19.0%, and 17.8% for respective time values of 41.2, 38.4, and 36.2 milliseconds. B, Plot of slack step vs duration of unloaded shortening yielded unloaded shortening velocity values of 1.86 muscle lengths (ML)/s in the control cell, 2.62 ML/s in the ISO-treated cell, and 2.91 ML/s in the PKA-treated cell. Inset: Tension-pCa relations were plotted for the same three cells.
43.2±7.7 kN/m² in PKA-treated cells. The higher overall force during the PKA experiments, which were conducted after the ISO experiments, was most likely due to refinements in isolation and attachment techniques over time, which yielded more robust cells.

PKA treatment resulted in a significant increase in the 3P content of TnI and C protein (identified by their relative positions on the Coomassie-stained gel) (Fig 7). These results are in agreement with previous studies showing that β-adrenergic stimulation and PKA treatment result in phosphorylation of these two proteins.

**Discussion**

The present study investigated mechanisms by which phosphorylation of myofilament proteins modulates the contractility of cardiac muscle during β-adrenergic stimulation. At the molecular level, contractility is ultimately determined by two variables: the number of activated crossbridges and the rate at which they cycle.27 A potent modulator of both variables in intact myocardium is the substantial alteration in the dynamics of Ca²⁺ handling by the sarcomegma and sarcoplasmic reticulum that occurs as a result of β-receptor stimulation.10 Larger Ca²⁺ transients during a twitch increase activation of the thin filaments, leading to a greater number of active crossbridges and more force.16 In addition, Vₚ was shown to increase with [Ca²⁺] in a variety of cardiac preparations.7,11,28

Our results indicate that β-adrenergic receptor-mediated phosphorylation of myofilament proteins also leads to functional changes that affect both crossbridge recruitment and cycling rate: the Ca²⁺ sensitivity of tension is reduced, and the rate of crossbridge detachment from actin is increased.

Stimulating membrane receptors with ISO, followed by rapid skinning, reduced the Ca²⁺ sensitivity of isometric tension (Fig 3 and Table 1), in agreement with previous results.14 Taken together with earlier work demonstrating that β-receptor stimulation reduces Ca²⁺ sensitivity in intact cardiac preparations,15,16 this result suggests that the skinning protocol effectively fixes myofilament proteins in a stimulated state. Furthermore, incubating skinned control cells with PKA caused a similar reduction in Ca²⁺ sensitivity (Fig 4), indicating that the effects of β-receptor stimulation are mediated by phosphorylation of myofilament protein(s) as a result of activation of the adenyl cyclase–cAMP pathway. The autoradiography results (Fig 7) more specifically suggest that TnI and/or C protein are the proteins of interest. TnI was previously implicated in mediating a reduction in Ca²⁺ sensitivity of tension, since its phosphorylation in vitro reduces the affinity of troponin C for Ca²⁺.25 It has been suggested that a reduction in Ca²⁺ sensitivity could underlie the increased rate of twitch relaxation during β-adrenergic stimulation by accelerating inactivation of the thin filament at the end of a contraction.29

The rate of crossbridge cycling could also influence twitch kinetics in intact myocardium, and our Vₚ results indicate that β-adrenergic-mediated phosphorylation of TnI and/or C protein increases that rate. This finding agrees with results from experiments that demonstrated a β-agonist–induced increase in the frequency of fₘin in papillary muscles during Ba²⁺ contracture.23 In contrast to other results from intact multicellular preparations that showed no effect of β-adrenergic stimulation on crossbridge cycling rate,6,27 the measurements of fₘin were performed during steady near-maximal Ba²⁺ contracts, thereby eliminating possible confounding effects due to changes in Ca²⁺ handling during β-adrenergic stimulation. Of particular interest with respect to the present results, the increase in fₘin (from 16% to 49%) was similar to the increases we observed in Vₚ in ISO-treated (38%) and in PKA-treated (41%) cells. This agreement is somewhat surprising in light of the fact that the fₘin studies were conducted under isometric conditions, whereas our experiments measured maximum Vₚ under unloaded conditions. Because mean crossbridge strain should be different in the two measurements, agreement in the results suggests that β-adrenergic–induced increases in the rate of crossbridge cycling do not vary with crossbridge strain.

An increase in the rate of crossbridge detachment could in part be responsible for β-receptor–mediated increases in twitch tension and the rates of force development and relaxation in intact cardiac muscle but would not be expected to increase the maximum isometric force. In this regard, our data suggest that the maximal force per cross-sectional area is reduced. This may be due to a decrease in the proportion of time the rapidly cycling crossbridges spend in strongly bound states. Brandt et al have suggested that changes in cycling rate may contribute to changes in Ca²⁺ sensitivity by modulating the cooperative activation of the thin filament.

The relative importance of TnI and C-protein phosphorylations in mediating the increase in cycling rate is not known at present. In skinned skeletal muscle fibers, extracting C protein increases velocity in the low-velocity phase of unloaded shortening observed at submaximal Ca²⁺.31 When it is assumed that compressive strain of a slowly detaching population of crossbridges limits shortening velocity at low Ca²⁺,32 this result is consistent with the hypothesis that C protein modulates crossbridge interaction by restricting the range of movement of crossbridges.31 It is possible that C-protein phosphorylation modulates this function, as suggested by the correlation observed between C-protein phosphorylation and the rate of relaxation in amphibian heart.33 On the other hand, experiments demonstrating that Vₚ varies with
[Ca\(^{2+}\)]\(_{11,28}\) show that the crossbridge cycling rate can be modulated by varying thin-filament activation; thus, it seems reasonable to suppose that TnI phosphorylation also might influence cycling kinetics because of its close association with troponin C in the thin filament. Distinguishing the relative importance of TnI and C protein in modulating the crossbridge cycling rate will require further investigation.

**Acknowledgments**

This study was supported by grant HL-25861 from the National Institutes of Health (Dr Moss) and a Wisconsin Heart Association Predoctoral Fellowship (Dr Strang). The authors thank Scott Stoker and Shermini Saini for their assistance with the experimental preparation and Dr James Graham for performing the SDS-PAGE on single myocytes.

**References**


32. Moss RL. Variations in maximum shortening velocity in skinned skeletal muscle fibers due to changes in thin filament activation with Ca++ and by partial extraction of troponin-C. *J Physiol (Lond.)* 1986;377:487-505.

Beta-adrenergic receptor stimulation increases unloaded shortening velocity of skinned single ventricular myocytes from rats.

K T Strang, N K Sweitzer, M L Greaser and R L Moss

Circ Res. 1994;74:542-549
doi: 10.1161/01.RES.74.3.542

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/3/542