Lack of Effect of Isoproterenol on Unloaded Velocity of Sarcomere Shortening in Rat Cardiac Trabeculae

Pieter P. de Tombe and Henk E.D.J. ter Keurs

Several recent reports have indicated that catecholamines may act directly on the crossbridge cycle, independent of intracellular calcium concentration changes. The present study investigated the effect of isoproterenol on peak force during twitches at constant sarcomere length and unloaded velocity of sarcomere shortening in isolated right ventricular trabeculae of hearts with V1 or V2 isomyosin obtained from euthyroid and hypothyroid rats, respectively. Hypothyroidism was induced by treatment of the rats with propylthiouracil for 6 weeks. Electrophoretic analysis showed that the hearts of hypothyroid animals were composed only of V2 isomyosin, whereas the hearts of euthyroid animals were composed predominantly of V1 isomyosin. Force development was measured with a silicon strain gauge and sarcomere length with laser diffraction techniques; the shortening velocity was determined from contractions in which sarcomere length was initially held constant followed by a quick release to zero load and a controlled release at zero load. Both isometric twitch force and unloaded sarcomere shortening velocity were sigmoidal functions of [Ca^{2+}]_o and of the concentration of isoproterenol. At optimal [Ca^{2+}]_o, unloaded shortening velocity was 40% lower in myocardium of hypothyroid animals than in myocardium of euthyroid animals. Isoproterenol increased the sensitivity of isometric twitch force and unloaded shortening velocity to [Ca^{2+}]_o in trabeculae from both euthyroid and hypothyroid animals. Isoproterenol did not increase unloaded shortening velocity at optimal [Ca^{2+}]_o regardless of the thyroid state. From these results we conclude that β-adrenergic stimulation per se does not accelerate the rate limiting step in the crossbridge cycle that determines unloaded sarcomere shortening velocity in the intact cardiac cell. (Circulation Research 1991;68:382–391)

Recent studies of stiffness of intact1–4 and chemically skinned5,6 cardiac papillary muscle preparations by stepwise and sinusoidal length perturbations have suggested that β-adrenergic stimulation increases the cycling rate of crossbridges during force generation at constant muscle length, independent of changes in the level of contractile activation. A similar result has been reported by Winegrad6 and Winegrad and Weisberg7 using EGTA-treated muscle. With this preparation, an increase in maximum calcium-activated force development and myosin ATPase activity was observed as a result of β-adrenergic stimulation.

It seems logical to conclude from the observation that the cycling rate of crossbridges is enhanced by isoproterenol that the unloaded shortening velocity of the muscle should increase. However, direct measurement of the effect of β-adrenergic stimulation on maximum shortening velocity indicated that it does not.8 A possible resolution for the difference in conclusions from the study on maximal velocity and those on oscillatory power1–7 is that the latter studies used predominantly muscles with V1 myosin isoenzyme, whereas the former study8 used principally muscles with V3 isoenzyme. To date, no studies have been reported in which the unloaded velocity of sarcomere shortening has been assessed in response to β-adrenergic stimulation. This measurement is essential, because extrapolation of the force–muscle length velocity relation to zero load to obtain un-

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loaded sarcomere shortening velocity is fraught with uncertainty.\textsuperscript{9-11} Furthermore, we previously have found that not only isometric twitch force but also unloaded sarcomere shortening velocity is a function of \([\text{Ca}^{2+}]_o\).\textsuperscript{9,10} Because calcium handling by the cardiac cell is profoundly altered on \(\beta\)-adrenergic stimulation,\textsuperscript{12} it is important to measure unloaded sarcomere shortening velocity under conditions at which velocity is independent of \([\text{Ca}^{2+}]_o\).

The aim of the present study, therefore, was to investigate which parameters of the relations between unloaded shortening velocity and \([\text{Ca}^{2+}]_o\), and between twitch force and \([\text{Ca}^{2+}]_o\), are affected by \(\beta\)-adrenergic stimulation. Sarcomere length was measured by laser diffraction methods and sarcomere shortening velocity by the isovelocity release technique\textsuperscript{9-11} in trabeculae from the right ventricle of the rat. These techniques allowed direct measurement of sarcomere shortening velocity, which, as was pointed out above, is essential in the study of cardiac muscle twitch kinetics.\textsuperscript{9-11} Because peak twitch force at constant sarcomere length and unloaded sarcomere shortening velocity are the main parameters that will be evaluated in this study, we will henceforth refer to them as (twitch) force and (unloaded shortening) velocity.

Studies by Winegrad and Weissberg and Chiu et al\textsuperscript{7} have indicated that myocardium that contains predominantly \(V_1\) isomyosin responds to \(\beta\)-adrenergic stimulation, even at saturating concentrations of calcium. On the other hand, myocardium that contains predominantly \(V_2\) isomyosin did appear not to respond to \(\beta\)-adrenergic stimulation at a saturating calcium concentration.\textsuperscript{7,8} We therefore studied trabeculae from the hearts of both euthyroid and hypothyroid rats, which contain predominantly \(V_1\) and \(V_3\) myosin isoenzymes.

\(\beta\)-Adrenergic stimulation in the present study resulted in a marked increase of the sensitivity of both unloaded velocity and twitch force to extracellular calcium. Maximal unloaded sarcomere shortening velocity at optimal \([\text{Ca}^{2+}]_o\), however, was unaffected by isoproterenol in trabeculae dissected from both euthyroid and hypothyroid animals. These results indicate that \(\beta\)-adrenergic stimulation per se does not increase the rate of the step in the crossbridge cycle that determines unloaded shortening velocity in the intact cardiac cell.

**Materials and Methods**

**Muscle Preparation and Experimental Apparatus**

Sprague-Dawley rats, of either sex and fed ad libitum, were anesthetized with diethyl ether, and the hearts were rapidly excised. After excision, the heart was immediately perfused with a modified Krebs-Henseleit solution and placed in a dissection dish beneath a binocular microscope (Nikon SMZ-1, Tokyo) equipped with an ocular micrometer (15-\(\mu\)m resolution). Thin, unbranched, and uniform trabeculae running between the free wall of the right ventri-
1. Briefly, sarcomere length was kept constant at 2.1–2.2 μm during the initial phase of the twitch by stretching the trabecula from the valvular end (panel B). Unloaded velocity is independent of sarcomere length in the range 1.9–2.2 μm.9–11 When about 50–70% of twitch force was attained, the muscle was quickly released to zero load, followed by a controlled, isovelocity release. Unloaded sarcomere velocity is independent of the time of release after about 40 msec into the twitch at 25°C, which coincides with approximately 50% force level.9 The speed and amplitude of this release were adjusted such that force remained constant. During the first 0.15 μm of sarcomere length shortening, velocity was measured by linear regression of the digitized sarcomere values (see Figure 1C) with respect to time. Peak twitch force was measured at constant sarcomere length (see Figure 1B).

**Induction of Hypothyroidism and Myosin Isoenzyme Distribution**

Hypothyroidism was induced in rats by ingestion of 0.8 g/l propylthiouracil (PTU) for 6 weeks via the drinking water.2,3,13,14 Starting at the age of 6 weeks. Control rats, also from the time of 6 weeks of age, were kept under identical conditions but were not treated. Both groups received water and food ad libitum. After excision of the heart, blood samples were collected for the determination of serum thyroxine (T₄) levels. Serum samples were frozen at −20°C for less than 4 months until analysis of T₄ levels by radioimmunoassay.

After dissection, all hearts were frozen and stored in liquid nitrogen until analysis. Myosin isoenzyme distribution was analyzed by pyrophosphate gel electrophoresis according to the method described by Hoh.
et al.\textsuperscript{15} The relative contribution of isomyosin was estimated by laser density scans of the gels. V\textsubscript{1} and V\textsubscript{3} areas were calculated assuming that the peaks were symmetric. If the peaks of V\textsubscript{1} or V\textsubscript{3} were asymmetric, it was assumed that the asymmetry resulted from the contribution of either non-V\textsubscript{1} or non-V\textsubscript{3} isoenzyme, respectively.\textsuperscript{14} A mixture of extracts of the hearts of a euthyroid and hypothyroid animal was always included as a calibration for the position of the V\textsubscript{1} and V\textsubscript{3} isomyosin in the test samples.

Statistical Analysis

Sigmoidal relations were fitted by a nonlinear fit procedure (Marquardt) to a modified Hill equation:

$$Y = b + a \cdot \frac{X^h}{(X^h + EC_{50}^h)}$$

where Y is the dependent variable, that is, force or unloaded shortening velocity; a represents the maximum saturated value Y can attain; b is a variable offset only used when appropriate (i.e., isoproterenol dose–response curve) and fixed to zero in all other cases; EC\textsubscript{50} is the value of X (i.e., the concentration of isoproterenol or [Ca\textsuperscript{2+}]) at which Y is 50% of parameter a and represents a compound affinity constant; and h represents the slope of the system (the Hill coefficient).

Two-way analysis of variance was used to compare EC\textsubscript{50} for [Ca\textsuperscript{2+}] and for isoproterenol; in this test the data were grouped as thyroid status, twitch force, and shortening velocity. Unpaired Student's t test was used to compare all parameters of the euthyroid and the PTU-treated group.\textsuperscript{16} Also, two-way repeated measures of analysis of variance were performed, in which data were grouped as to whether isoproterenol was present or not, and force or velocity, to test for the effect of isoproterenol and possible interaction of this effect on either force or velocity. The fit parameters that resulted from the nonlinear fit procedure were subjected to these statistical tests as if they had been obtained from direct measurements. Results are expressed as mean±SEM unless indicated otherwise. Values of p<0.05 were considered significant.

**Results**

**Effects of Hypothyroidism**

PTU treatment resulted in a significant decline in T\textsubscript{4} level at the time the animal was killed, from 53±3.5 nM in euthyroid animals to a nondetectable level in PTU-treated animals (less than 7 nM). Concomitantly, body weight and heart weight were significantly lower in the PTU-treated animals (see Figure 2A, top graphs). Both time to peak twitch force and relaxation were significantly prolonged in the muscle preparations dissected from hypothyroid animals (Figure 2A, bottom left graph). Cardiac isomyosin composition was shifted toward V\textsubscript{3} isomyosin in hypothyroidism (Figure 2B). We examined both left and right ventricles of euthyroid (n=18) and hypothyroid (n=18) animals and could not detect V\textsubscript{1} isomyosin in the hypothyroid group, whereas V\textsubscript{1} was approximately 90% in the euthyroid animals.
The effect of hypothyroidism on maximal twitch force at saturating [Ca$^{2+}$]$_0$, (i.e., greater than 2.5 mM) is shown in the bottom right graph of Figure 2A. Maximal twitch force was significantly lower (40%) in hypothyroidism. We consider this difference as real, even though the calculation of cross-sectional area from microscopical measurements (accuracy, 10 μm for width and 15 μm for thickness) is relatively inaccurate. Hence, to investigate the effects of varied concentrations of external calcium and isoproterenol more precisely, we normalized twitch force to maximum twitch force, at saturating [Ca$^{2+}$]$_0$ in each individual experiment.

Sensitivity of Isometric Twitch Force and Unloaded Sarcomere Shortening Velocity to Isoproterenol

We first determined the sensitivity of twitch force and unloaded shortening velocity to isoproterenol in four muscles to establish the concentration of isoproterenol at which force and velocity were saturated. An example of the response of force and velocity to increasing concentrations of isoproterenol is shown in Figure 3B. We chose a [Ca$^{2+}$]$_0$ of 0.3 mM for these experiments, a concentration close to the EC$_{50}$ of [Ca$^{2+}$], for the velocity of unloaded shortening and slightly below the EC$_{50}$ for twitch force (see Table 2), to allow a maximal response to isoproterenol. The response of force and velocity to isoproterenol were fitted to Equation 1, to which a variable offset had been added, and the average fit parameters are shown in Table 1. The EC$_{50}$ of isoproterenol for velocity and force was higher in hypothyroidism, as has been observed before. Unloaded shortening velocity, at a saturating isoproterenol concentration, was 44% lower in hypothyroidism and did not differ from the maximal velocity determined in the absence of isoproterenol (see Table 2). Twitch force, at a [Ca$^{2+}$], of 0.3 mM and at a saturating isoproterenol concentration, was 25–30% lower with isoproterenol than maximal force in the absence of the drug (see Table 2).

Effect of [Ca$^{2+}$]$_0$ on Isometric Twitch Force and Unloaded Sarcomere Shortening Velocity

Shortening velocity and twitch force were both sigmoidal functions of [Ca$^{2+}$]$_0$ as shown in Figure 4 for a representative trabecula of each of the thyroid groups in the absence of isoproterenol. The data were fitted to Equation 1, and the fitted line is indicated in Figure 4. The average parameters of the fit are shown in Table 2. Two-way analysis of variance revealed that the EC$_{50}$ of [Ca$^{2+}$]$_0$ for force was significantly higher (p<0.01) than the EC$_{50}$ for velocity in both thyroid groups, which is consistent with previous findings from this laboratory.9,10

### TABLE 1. Isoproterenol Dose–Response Curve

<table>
<thead>
<tr>
<th>V$_0$</th>
<th>F$_0$</th>
<th>Maximum (μm/sec)</th>
<th>EC$_{50}$ (nM)</th>
<th>Maximum (%)</th>
<th>EC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid (n=7)</td>
<td>12.6±0.52</td>
<td>6.9±1.2</td>
<td>73±6.7</td>
<td>11.7±3.0</td>
<td></td>
</tr>
<tr>
<td>PTU treated (n=4)</td>
<td>7.0±0.60*</td>
<td>17.1±3.3*</td>
<td>54±4.1</td>
<td>15.0±1.9*</td>
<td></td>
</tr>
</tbody>
</table>

Average parameters of relation between unloaded sarcomere shortening velocity (V$_0$) and isometric twitch force (F$_0$) and concentration of isoproterenol, at [Ca$^{2+}$]$_0$. Values are mean±SEM. PTU, propylthiouracil.

*p<0.05, euthyroidism vs. PTU treated.
velocity was 40% lower in trabeculae dissected from hypothyroid animals, as also shown in Figure 1C. The EC₅₀ of [Ca²⁺]₀ for velocity and force, however, was independent of the thyroid status of the animals from which the trabeculae had been dissected.

**Effect of Isoproterenol on the Force–[Ca²⁺]₀ and Velocity–[Ca²⁺]₀ Relations**

From the results presented in Figure 3 and Table 1, it is apparent that both twitch force and unloaded velocity were a function of the isoproterenol concentration and were saturated at isoproterenol concentrations less than 100 nM. Hence, we studied the force–[Ca²⁺]₀ and velocity–[Ca²⁺]₀ relations further at a saturated level of β-adrenergic stimulation, that is, 100 nM isoproterenol. Both velocity and force were again sigmoidal functions of [Ca²⁺]₀, and the average fit parameters are shown in Table 2.

The sensitivity of velocity and force to [Ca²⁺]₀ were markedly increased (60–80%) in the presence of isoproterenol. It is also noteworthy that the sensitivity of force to [Ca²⁺]₀ was always higher than that of velocity. The latter observation is consistent with the difference in sensitivity of force compared with that of velocity to [Ca²⁺]₀ in the absence of isoproterenol.

Table 2 shows that regardless of the thyroid state, maximal unloaded velocity was unaffected by isoproterenol. Maximal twitch force, on the other hand, was significantly reduced in the presence of isoproterenol. The majority of preparations developed spontaneous contractions and triggered arrhythmias at a [Ca²⁺]₀ above 1.5 mM in the presence of 100 nM isoproterenol.

**TABLE 2. Extracellular Calcium Concentration Dose–Response Curve**

<table>
<thead>
<tr>
<th></th>
<th>Maximum V₀ (µm/sec)</th>
<th>EC₅₀ (mM)</th>
<th>Maximum F₀ (%)</th>
<th>EC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ISO (n=7)</td>
<td>13.3±0.33</td>
<td>0.34±0.02</td>
<td>100</td>
<td>0.44±0.08*</td>
</tr>
<tr>
<td>+ ISO (n=6)</td>
<td>13.2±0.53</td>
<td>0.07±0.11†</td>
<td>68±3.9†</td>
<td>0.09±0.01††</td>
</tr>
<tr>
<td>PTU treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ISO (n=5)</td>
<td>7.9±0.22‡</td>
<td>0.29±0.04</td>
<td>100</td>
<td>0.49±0.06*</td>
</tr>
<tr>
<td>+ ISO (n=5)</td>
<td>7.4±0.39‡</td>
<td>0.12±0.02‡†</td>
<td>75±5.8†</td>
<td>0.19±0.04††</td>
</tr>
</tbody>
</table>

Average parameters of relation between unloaded sarcomere shortening velocity (V₀) and isometric twitch force (F₀) and [Ca²⁺]₀ with (+) and without (−) isoproterenol (ISO) (100 nM) in euthyroid and hypothyroid trabeculae. Values are mean±SEM. PTU, propylthiouracil.

*p<0.01, F₀ vs. V₀.

†p<0.05, −ISO vs. +ISO.

‡p<0.05, euthyroid vs. PTU treated.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4. Relations between [Ca²⁺]₀ and unloaded shortening velocity (V₀) and isometric twitch force (F₀).** Panels A and C show results obtained in a trabecula from a euthyroid animal; panels B and D show the results from a trabecula dissected from a hypothyroid animal. Relations were determined as described in text. Data were fitted to a modified Hill equation, which resulted in the parameters EC₅₀ and maximal V₀, and maximal F₀, as indicated by the dashed arrows. Fit parameters were: (panel A) r=0.998; n=85; EC₅₀=0.41 mM; h=2.8; maximal V₀=14.2 µm/sec; (panel B) r=0.993; n=43; EC₅₀=0.30 mM; h=3.1; maximal V₀=7.9 µm/sec; (panel C) r=0.999; n=75; EC₅₀=0.52 mM; h=2.2; maximal F₀=100%; (panel D) r=0.994; n=70; EC₅₀=0.48 mM; h=3.2; maximal F₀=100%.
isoproterenol, at which time the experiments were terminated. However, the high sensitivity of the unloaded shortening velocity and twitch force (i.e., EC₅₀<0.3 mM; see Table 2) for [Ca²⁺]ₐ, resulted in saturation of both velocity and force at [Ca²⁺]ₐ less than 1 mM, if the muscles were exposed to 100 nM isoproterenol. Hence, the assessment of saturated unloaded sarcomere shortening velocity and isometric twitch force, as well as the EC₅₀ for [Ca²⁺]ₐ, was unaffected by spontaneous activity.

Exposure of the muscles to isoproterenol reduced maximal twitch force, which by itself could have decreased the velocity. This effect cannot have been large, because in several muscles the reduction of maximal twitch force by isoproterenol was less than 20% (e.g., Figure 3), whereas unloaded shortening velocity was not increased above the maximal velocity without isoproterenol. We assessed the interaction between twitch force and unloaded shortening velocity at varied [Ca²⁺]₀ and at varied isoproterenol concentrations; Figure 5 shows this relation in both thyroid groups. It is clear that shortening velocity increased in proportion to twitch force below 40% of maximal force. The increase of velocity at higher force levels, however, is substantially smaller, as was expected from the differences between the sensitivities of velocity and force for [Ca²⁺]₀ shown in Table 2.

The change of unloaded shortening velocity with twitch force was less than 2% of the maximal velocity for a 10% increment of force between 60% and maximal force. Linear regression through the data above 60% maximal force failed to show significance of correlation between velocity and force in either thyroid group, as we have reported before for euthyroid trabeculae. Also, fitting a second-order polynomial through the complete twitch force–unloaded velocity relation indicated an increase less than 6% of maximal velocity for an increase of force from 70% to 100%.

Discussion

The general properties of the two groups of muscles studied here were comparable with those that have been reported previously in the literature. Ninety percent of cardiac myosin consisted of V₁ isoenzyme in euthyroid animals (see Figure 2). The PTU-treated animals were hypothyroid, as was shown by their plasma T₄ levels, reduced body weight, and slower twitch kinetics (see Figure 2). These effects have been reported before and are consistent with the complete conversion of the expression of myosin isoenzyme to the V₁ form (Figure 2) by PTU treatment. Force development corrected for the cross-sectional area of the muscles was on average smaller in the hypothyroid group than in the euthyroid muscles, as has been observed in earlier studies. The questions need further analysis of whether this difference is due to 1) an altered fraction of attached crossbridges at the moment of peak force, 2) an alteration of the level of activation of the contractile filaments by Ca²⁺ ions as a result of an altered thyroid state, or 3) both of these possibilities. The observation that the sensitivities of unloaded shortening velocity and twitch force for isoproterenol were lower in hypothyroidism is also consistent with previous reports in the literature and probably is due to a reduced number of myocardial β-receptors as a result of hypothyroidism.

The maximum shortening velocity was reduced in muscles of the hypothyroid animals as has been observed previously in myocardium of rat, cat, and rabbit. The difference between unloaded shortening velocity in the muscles with predominantly V₁ isoenzyme and those with V₁ isoenzyme (a factor of approximately 2) in this study was smaller than that described previously (a factor of approximately 6). This observation deserves further comment. It is possible that the difference relates to the method of measurement of shortening velocity (unloaded shortening velocity of sarcomeres in this study versus unloaded shortening of the muscle by Pagani and Julian). Alternatively, the discrepancy could be due to large differences in the level of activation between euthyroid and hypothyroid muscles; we have eliminated this factor by studying shortening velocity at a wide range of [Ca²⁺]₀. Lastly, it is possible that the difference reflects different properties of myosin in rat cardiac muscle versus those in rabbit.

The purpose of the induction of hypothyroidism by means of PTU in this study was to compare the effects of isoproterenol on the dynamic properties of the contractile system of cardiac muscle with V₁ myosin isoenzyme (our controls) with those on muscles containing only V₃ isoenzyme (the PTU group). Table 2 shows that a saturating concentration of
isoproterenol (0.1 μM) reduced twitch force maximum by approximately 30%, whereas it did not change unloaded sarcomere shortening velocity at all. The lack of effect on unloaded shortening velocity is striking because previous studies have concluded that the rate of crossbridge cycling increases 25–50% in response to isoproterenol (0.3–6 μM) in rat3,5 and rabbit.1 Therefore, one might have expected an increase of the shortening velocity of the same order of magnitude. Such an increase was neither observed in a previous study on the velocity of muscle shortening6 nor in this study.

The lack of effect of isoproterenol on unloaded sarcomere shortening velocity could have resulted from a reduction of twitch force by changes in Ca2+ metabolism of the myocyte due to isoproterenol. Hence, we analyzed the effects of [Ca2+]c and isoproterenol on twitch force and shortening velocity to assess how much isoproterenol could have reduced shortening velocity by its effects on twitch force. Figure 5 shows that an increase in twitch force induced by [Ca2+]c and isoproterenol up to 40% of maximal force is accompanied by a steep increase of the velocity. At higher forces the rise in velocity is much less, so that variation of force between 70% and maximal force was accompanied by a negligible variation of velocity (less than 6%). A more accurate prediction of the effect of a decrease of maximal force due to isoproterenol on unloaded sarcomere shortening velocity might be obtained with a description of the complete relation between unloaded velocity and isometric force. Such a description requires a model of the mechanism that relates the activation level of the contractile filaments by calcium ions to unloaded shortening velocity. The latter mechanism is the subject of considerable, but unresolved, discussion.24 We have not tried to investigate this complex issue in the present study, and we also have refrained from further analysis of the shape of the relation because the combined data in Figure 5 clearly show that 25–30% reduction of maximal twitch force by isoproterenol cannot have obscured a significant (i.e., more than 6%) increase of unloaded shortening velocity. This conclusion is reinforced by the observation (see, for example, Figure 3) that when isoproterenol little reduced maximal twitch force (e.g., 10–20%), as it did in several muscles from both groups, maximal velocity was not increased at all above the value measured without isoproterenol.

The results of the experiments on rat cardiac trabeculae described here clearly suggest that the latter hypothesis is untenable. This discrepancy raises two intriguing questions. First, which mechanism of operation of the actin-myosin interaction can explain that isoproterenol accelerates the kinetics of isometric force generation but fails to increase the unloaded shortening velocity? Second, is the absence of an effect of β-adrenergic stimulation on unloaded sarcomere shortening velocity a unique property of rat myocardium, or can this phenomenon be expected in cardiac muscle of other species?

The different responses of force dynamics and of shortening velocity possibly can be explained by assuming that different steps in the crossbridge cycle are rate limiting for force generation and for rapid unloaded propulsion of the actin filament. Similarly, Siemankowski et al26 have proposed that of the different reaction steps of myosin ATPase, one step is rate limiting for unloaded shortening and another limits the ATPase activity in vitro. Both steps may be affected by modification of the myosin isoenzyme composition, but not necessarily by β-adrenergic stimulation. It is likely that the frequency at which stiffness of the activated muscle is minimal corre-
sponds to the rate of force development, but it is not necessary that this property relates to the unloaded shortening velocity. First, the rate constant of cross-bridge cycling generally is assumed to depend on the strain of the crossbridge, which evidently is high during the test of stiffness. It also is likely that during sinusoidal perturbations, crossbridges go through a complete cycle of ATP hydrolysis. On the other hand, the unloaded shortening velocity probably depends on one particular fast reaction step in the crossbridge cycle. In fact, a simple calculation shows that the unloaded shortening velocity predicted from the frequency of the stiffness minimum (approximately 1 Hz) and the stroke of a crossbridge is about two orders of magnitude too low. Therefore, unloaded shortening velocity and stiffness minimum are not a priori expressions of the same molecular reaction processes.

The sensitivity of rat myocardium to \([\text{Ca}^{2+}]_o\) is higher than myocardium of other mammals. We recently have shown, however, that the effects of \([\text{Ca}^{2+}]_o\) on the force–sarcomere length relation and force–sarcomere velocity relation of cat cardiac trabeculae are in several ways similar to those in the rat. The main differences between rat and cat myocardium in that study were slower twitch kinetics and reduced \([\text{Ca}^{2+}]_o\) sensitivity. The similarities between rat and cat cardiac trabeculae suggest that the second question mentioned above can be answered by using cat cardiac trabeculae to test the hypothesis that \(\beta\)-adrenergic stimulation increases the level of activation and thereby increases unloaded shortening velocity in mammalian cardiac muscle.

An increase in force development and ATPase activity on \(\beta\)-adrenergic stimulation or cyclic AMP application at a saturating calcium concentration has been reported in EGTA-treated cardiac muscle. The isomyosin composition appeared to have a major influence on the magnitude of the force response to \(\beta\)-adrenergic stimulation or cyclic AMP at saturating calcium concentrations. This effect, however, has not been observed in other studies. In the present study, the response of twitch force to isoproterenol was not affected by the thyroid status. It is not clear what the reason is for these differences. They could be due to the large difference in the preparations used, that is, EGTA-treated fibers versus intact trabeculae in this study.

In conclusion, \(\beta\)-adrenergic stimulation does increase the unloaded velocity of sarcomere shortening and twitch force under physiological conditions, such as in the intact animal at submaximal levels of \([\text{Ca}^{2+}]_o\). This effect, however, is due to a greater sensitivity of unloaded shortening velocity to extra- cellular \(\text{Ca}^{2+}\), presumably as a result of larger \(\text{Ca}^{2+}\) influx into the cardiac cell and hence larger \(\text{Ca}^{2+}\) release from intracellular stores, and not due to an intrinsic modification of the rate limiting step in the actomyosin interaction that determines unloaded shortening velocity of cardiac muscle.

Acknowledgments

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KEY WORDS • isoproterenol • force–sarcomere velocity relation • hypothyroidism • isomyosin
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