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 $V_1$ or $V_3$ 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 $V_3$ isomyosin, whereas the hearts of euthyroid animals were composed predominantly of $V_1$ 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 $[Ca^{2+}]_{i}$, and of the concentration of isoproterenol. At optimal $[Ca^{2+}]_{i}$, 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 $[Ca^{2+}]_{i}$ in trabeculae from both euthyroid and hypothyroid animals. Isoproterenol did not increase unloaded shortening velocity at optimal $[Ca^{2+}]_{i}$ regardless of the thyroid state. From these results we conclude that $\beta$-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 intact cardiac papillary muscle preparations by stepwise and sinusoidal length perturbations have suggested that $\beta$-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 Winegrad and Winegrad and Weisberg 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 $\beta$-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 $\beta$-adrenergic stimulation on maximum shortening velocity indicated that it does not. A possible resolution for the difference in conclusions from the study on maximal velocity and those on oscillatory power is that the latter studies used predominantly muscles with $V_1$ myosin isoenzyme, whereas the former study used principally muscles with $V_3$ isoenzyme. To date, no studies have been reported in which the unloaded velocity of sarcomere shortening has been assessed in response to $\beta$-adrenergic stimulation. This measurement is essential, because extrapolation of the force–muscle length velocity relation to zero load to obtain un-
load sarcomere shortening velocity is fraught with uncertainty.9–11 Furthermore, we previously have found that not only isometric twitch force but also unloaded sarcomere shortening velocity is a function of \([Ca^{2+}]_o\).9,10 Because calcium handling by the cardiac cell is profoundly altered on \(\beta\)-adrenergic stimulation,12 it is important to measure unloaded sarcomere shortening velocity under conditions at which velocity is independent of \([Ca^{2+}]_o\).

The aim of the present study, therefore, was to investigate which parameters of the relations between unloaded shortening velocity and \([Ca^{2+}]_o\), and between twitch force and \([Ca^{2+}]_o\), are affected by \(\beta\)-adrenergic stimulation. Sarcomere length was measured by laser diffraction methods and sarcomere shortening velocity by the isovelocity release technique9–11 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.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 Weisberg7 and Chiu et al8 have indicated that myocardium that contains predominantly V1 isomyosin responds to \(\beta\)-adrenergic stimulation, even at saturating concentrations of calcium. On the other hand, myocardium that contains predominantly V3 isomyosin did appear not to respond to \(\beta\)-adrenergic stimulation at a saturating calcium concentration.7,8 We therefore studied trabeculae from the hearts of both euthyroid and hypothyroid rats, which contain predominantly V1 and V3 myosin isoenzymes.

\(\beta\)-Adrenergic stimulation in the present study resulted in a marked increase in the sensitivity of both unloaded velocity and twitch force to extracellular calcium. Maximal unloaded sarcomere shortening velocity at optimal \([Ca^{2+}]_o\), 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-

... and the tricuspid valve were dissected carefully. The dimensions of the preparations were 2–5 mm in length, 50–200 \(\mu\)m in width, and 40–80 \(\mu\)m thick. The trabeculae were attached at one end to the remnant of the tricuspid valve and at the other end to a block cut from the right ventricular wall.

The muscle preparations were mounted in an experimental chamber positioned on an inverted microscope (model Diaphot-TMD, Nikon) as described previously.11 The microscope was equipped with a television system (model TR930 monitor, model WV1500 camera, Panasonic, Saito, Japan) (final magnification, \(\times 100\)) to aid the measurement of muscle length and width (within 10 \(\mu\)m). The chamber was perfused with a modified Krebs-Henseleit solution at a flow rate of approximately 2.5 ml/min. Muscle preparations were electrically stimulated at 0.5 Hz via platinum electrodes; stimulus strength was adjusted to 50% above the stimulus threshold.

The standard solution used was a modified Krebs-Henseleit solution with the following composition (mM): \(Na^+\ 140.5, K^+\ 5.0, Cl^-\ 127.5, Mg^{2+}\ 1.2, H_2PO_4^-\ 2.0, SO_4^{2-}\ 1.2, HCO_3^- 19, d-glucose 10.0, and [Ca^{2+}]_o\) as indicated. All chemicals were of the highest purity available (Analar grade, BDH Chemical Co., Toronto). The solutions were in equilibrium with a 95% \(O_2\)-5% \(CO_2\) gas mixture, resulting in a pH of 7.4 at 25°C. The temperature in the perfusion chamber was controlled (±0.1°C) using a glass heat exchanger at the inflow line and a circulating water bath (model F3, Haake, Karlsruhe, FRG). Isoproterenol was infused at the inflow of the muscle bath to avoid oxidation of the drug. This procedure resulted in a steady-state level of increased twitch force in about 10 minutes.

The remnant of the tricuspid valve served as an attachment point to a servo-controlled motor used to control the length of the muscle preparation during the twitch.11 Force was measured with a modified silicon strain gauge,11 Sarcomere length was measured by laser diffraction techniques, as described in detail previously.9–11 Force, sarcomere length, and muscle length were displayed on a storage oscilloscope, recorded on a chart recorder, and sampled via an A/D converter installed in a personal computer.11

Potential sources of error associated with the use of laser diffraction techniques to measure sarcomere length or sarcomere shortening velocity are Bragg angle reflection artifacts and inhomogeneity of the muscle preparation. We recently have shown that the possible error in the measurement of sarcomere length and velocity that is due to these artifacts is less than 4% in our measurements.11

**Measurement of Unloaded Sarcomere Shortening Velocity and Isometric Twitch Force**

Maximum velocity of sarcomere shortening during the twitch was measured by means of an isovelocity release technique, as described previously9,10 and in detail.11 This method is illustrated in Figure
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. 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. 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, 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.

**FIGURE 1.** Measurement of isometric force development and unloaded sarcomere shortening velocity in rat cardiac muscle. Panels A and B: Twitch force (F), muscle length (ML), and sarcomere length (SL) measured in a representative euthyroid trabecula. Muscle length was held constant in panel A. Damaged ends of the preparation allowed substantial sarcomere shortening in the area of measurement during the twitch. Twitch timing characteristics were measured as illustrated by the dashed lines in panel A on the force recording. Time to peak force was defined as the time elapsed between 10% of peak force and peak force. Relaxation time was defined as the time elapsed between peak force and decay of force to 10% of peak force. In panel B, internal shortening was prevented during the first two thirds of the twitch by stretching the muscle by a servo motor attached to the valvular end. This procedure allowed the measurement of peak twitch force at constant sarcomere length (at about 2.05 μm). Panel C: Examples of the protocol used for the measurement of unloaded velocity of sarcomere shortening (VSL) in a euthyroid trabecula (top tracings) and hypothyroid trabecula (bottom tracings). Recordings show tracings of sarcomere length (SL) and force (F) during 30 msec of a release of SL at controlled velocity just before peak force of the twitch. SL was kept constant at 2.1–2.2 μm during the initial phase of the twitch, as in panel B. When F reached about 70% of peak twitch force, the preparation was released to zero force. V_SL was measured from the digitized SL recordings by computer within the time window indicated by the dashed lines. Top tracings show V_SL of 13.2 μm/sec in a trabecula from a euthyroid animal; bottom tracings show that V_SL is substantially lower (7.3 μm/sec) in a trabecula from a hypothyroid animal. [Ca²⁺]₀, 1.5 mM; temperature, 25°C; calibrations as indicated.
et al. The relative contribution of isomyosin was estimated by laser density scans of the gels. $V_1$ and $V_3$ areas were calculated assuming that the peaks were symmetric. If the peaks of $V_1$ or $V_3$ were asymmetric, it was assumed that the asymmetry resulted from the contribution of either non-$V_1$ or non-$V_3$ isoenzyme, respectively. A mixture of extracts of the hearts of a euthyroid and hypothyroid animal was always included as a calibration for the position of the $V_1$ and $V_3$ isomyosin in the test samples.

Statistical Analysis

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

$$Y = b + a \cdot \frac{X^h}{(X^h + EC_{50}^h)} \tag{1}$$

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; $EC_{50}$ is the value of $X$ (i.e., the concentration of isoproterenol or $[Ca^{2+}]_o$) 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 $EC_{50}$ for $[Ca^{2+}]_o$ 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. 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 $T_4$ 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 $V_1$ 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 $V_3$ isomyosin in the hypothyroid group, whereas $V_1$ was approximately 90% in the euthyroid animals.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Effects of hypothyroidism on twitch kinetics and myosin isoenzyme composition. Panel A: Effects of hypothyroidism on body weight (BW), heart weight (HW), time to peak force (TTP), time from peak force to 10% force during relaxation (RELAX) of the twitch at $[Ca^{2+}]_o$ of 1.0 mM, and peak isometric twitch force ($F_0$) at 2.0 μm sarcomere length and at saturating concentrations of $[Ca^{2+}]_o$ (>2.5 mM). EU, euthyroid animal; PTU, propylthiouracil-treated animal. Panel B: Photographs of electrophoresis gels with $V_1$ and $V_3$ isomyosin (top) and laser density scans of the gels (bottom). Arrows indicate the direction in which the proteins were separated. In the top panel, the gel at the right depicts a representative extract from the right ventricle of a euthyroid rat; the gel at the left shows a similar extract from a hypothyroid animal. The middle gel shows the separation of a mixture of extract from a euthyroid and hypothyroid animal and illustrates that the resolution of the separation is sufficient to resolve $V_1$ and $V_3$ isomyosin.
The effect of hypothyroidism on maximal twitch force at saturating [Ca\textsuperscript{2+}]\textsubscript{o}, (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 \mu m for width and 15 \mu m 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 maximal twitch force, at saturating [Ca\textsuperscript{2+}]\textsubscript{o}, 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\textsuperscript{2+}]\textsubscript{o} of 0.3 mM for these experiments, a concentration close to the EC\textsubscript{50} of [Ca\textsuperscript{2+}]\textsubscript{o} for the velocity of unloaded shortening and slightly below the EC\textsubscript{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\textsubscript{50} of isoproterenol for velocity and force was higher in hypothyroidism, as has been observed before.\textsuperscript{17,18} 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\textsuperscript{2+}]\textsubscript{o} 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\textsuperscript{2+}]\textsubscript{o} on Isometric Twitch Force and Unloaded Sarcomere Shortening Velocity**

Shortening velocity and twitch force were both sigmoidal functions of [Ca\textsuperscript{2+}]\textsubscript{o}, 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\textsubscript{50} of [Ca\textsuperscript{2+}]\textsubscript{o} for force was significantly higher (p<0.01) than the EC\textsubscript{50} for velocity in both thyroid groups, which is consistent with previous findings from this laboratory.\textsuperscript{9,10}

![Figure 3. Dependence of twitch force (F\textsubscript{o}) and unloaded shortening velocity (V\textsubscript{o}) on isoproterenol (ISO). Panel A: Effect of isoproterenol on sarcomere length (SL, top panel) and twitch force (F, bottom panel) in a trabecula dissected from a euthyroid animal; [Ca\textsuperscript{2+}]\textsubscript{o} was 0.3 mM. Isoproterenol (100 nM) was added at the time indicated by the arrow and subsequently was delivered to the muscle bath via a mixing pump. Calibrations: SL, 0.4 \mu m; F, 100 mg; time, 5 minutes. Resting sarcomere length, 2.2 \mu m; [Ca\textsuperscript{2+}]\textsubscript{o}, 1.0 mM. Panel B: Example of the F\textsubscript{o}–[ISO] relation. Panel C: Example of the V\textsubscript{o}–[ISO] relation in euthyroidism. Data were fitted to a modified Hill equation to which a variable offset had been added. The fit resulted in the parameters EC\textsubscript{50}, maximal V\textsubscript{o}, and maximal F\textsubscript{o}, as indicated by the dashed arrows; [Ca\textsuperscript{2+}]\textsubscript{o} was 0.3 mM. F\textsubscript{o} was normalized to the [Ca\textsuperscript{2+}]\textsubscript{o} saturated value observed in the absence of isoproterenol (see Figure 4). Maximal V\textsubscript{o} of this muscle at a saturating [Ca\textsuperscript{2+}]\textsubscript{o} was 14.2 \mu m/sec in the absence of isoproterenol. Fit parameters: (panel B) r=0.996; n=66; EC\textsubscript{50}=8.5 nM; h=1.9; maximal F\textsubscript{o}=81.4%; (panel C) r=0.988; n=75; EC\textsubscript{50}=12.2 nM; h=1.7; maximal V\textsubscript{o}=13.8 \mu m/sec.](http://circres.ahajournals.org/lookup/doi/10.1161/01.RES.68.2.386)
Effect of Isoproterenol in Rat Trabeculae

TABLE 2. Extracellular Calcium Concentration Dose–Response Curve

<table>
<thead>
<tr>
<th></th>
<th>Maximum (μm/sec)</th>
<th>EC50 (mM)</th>
<th>Maximum (%)</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.

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. It has also been shown that the effect of isoproterenol on velocity is synergistic with that of calcium concentration. The unloading effect of isoproterenol on velocity is dependent on calcium concentration. 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.

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 an 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., EC_{50}<0.3 mM; see Table 2) for [Ca^{2+}]_0, resulted in saturation of both velocity and force at [Ca^{2+}]_0 less than 1 mM, if the muscles were exposed to 100 nM isoproterenol. Hence, the assessment of saturated unloaded sarcomere shortening velocity and isotropic twitch force, as well as the EC_{50} for [Ca^{2+}]_0, 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 [Ca^{2+}]_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 [Ca^{2+}]_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 cor-

relation between velocity and force in either thyroid group, as we have reported before for euthyroid trabeculae.9 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.2,3,14 Ninety percent of cardiac myosin consisted of V_1 isoenzyme in euthyroid animals (see Figure 2). The PTU-treated animals were hypothyroid, as was shown by their plasma T_4 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 V_1 form (Figure 2) by PTU treatment.2,3,14 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.19 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 Ca^{2+} 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.18

The maximum shortening velocity was reduced in muscles of the hypothyroid animals as has been observed previously in myocardium of rat,21 cat,19,22 and rabbit.20 The difference between unloaded shortening velocity in the muscles with predominantly V_1 isoenzyme and those with V_3 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 [Ca^{2+}]_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 PTU2,3,14 in this study was to compare the effects of isoproterenol on the dynamic properties of the contractile system of cardiac muscle with V_1 myosin isoenzyme (our controls) with those on muscles containing only V_3 isoenzyme (the PTU group). Table 2 shows that a saturating concentration of
isoproterenol (0.1 \(\mu\text{M}\)) 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 \(\mu\text{M}\)) in rat\(^2\)\(^,\)\(^3\) 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 shortening\(^8\) 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 \(\text{Ca}^{2+}\) metabolism of the myocyte due to isoproterenol. Hence, we analyzed the effects of \(\left[\text{Ca}^{2+}\right]_c\) 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 \(\left[\text{Ca}^{2+}\right]_c\) 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 this study question the appealingly simple prediction that \(\beta\)-adrenergic stimulation should increase the unloaded shortening velocity due to the accelerated crossbridge cycling, as has been shown in studies on dynamic muscle stiffness and kinetics of force development.\(^1\)\(^–\)\(^3\)\(^,\)\(^25\)\(^,\)\(^28\)\(^–\)\(^31\) The prediction is based on analogy with the correspondence between unloaded velocity, dynamic muscle stiffness, and the biochemical properties of cardiac myosin isoenzymes.\(^1\)\(^–\)\(^3\)\(^,\)\(^25\)

It is well known that the ATPase activity of cardiac muscle depends on the amount of the myosin isoenzyme \(V_1\) relative to the amount of \(V_3\) myosin.\(^25\) That this biochemical parameter is reflected in the mechanical properties of cardiac muscle was shown by measurements of the dependence of stiffness on the frequency of small (less than 1%) length perturbations of cardiac muscle during constant activation by barium contractures. The frequency at which stiffness is minimal (approximately 1 Hz) appeared to correlate closely with the isomyosin composition of the muscle.\(^2\) Hence, the increase in frequency of minimal stiffness has been attributed to a higher cycling rate of \(V_1\) isomyosin than of \(V_3\) isomyosin.\(^1\)\(^–\)\(^3\)\(^,\)\(^25\) Similarly, a strong correlation between the unloaded shortening velocity and myocardial isomyosin composition has been demonstrated\(^20\)\(^,\)\(^21\)\(^,\)\(^23\) and is confirmed by the results of this study. Hence, the assumption that the frequency of minimal stiffness and unloaded shortening velocity are related expressions of the myosin ATPase activity is reasonable.

\(\beta\)-Adrenergic stimulation of cardiac muscle also has been shown to increase both myosin ATPase activity\(^6\)\(^,\)\(^7\) and the frequency of minimal stiffness in papillary muscles during barium contractures.\(^1\)\(^–\)\(^3\) The hypothesis that \(\beta\)-adrenergic stimulation induces an increase of the cycling rate of crossbridges was supported by the observation of Saeki et al.\(^4\)\(^,\)\(^5\) that catecholamines increased the rate of force redevelopment after small muscle length changes. They also concluded from their observations that increase of the crossbridge cycling rate by \(\beta\)-adrenergic stimulation was independent of the level of myofilament activation. Hence, by analogy to the correlation between the unloaded shortening velocity and myosin isoenzyme composition, it seems plausible to predict that \(\beta\)-catecholamines should increase unloaded shortening velocity irrespective of the level of activation of the muscle by calcium ions.

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 \(\beta\)-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 al.\(^26\) 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 \(\beta\)-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 \([Ca^{2+}]\) is higher than myocardium of other mammals. We recently have shown, however, that the effects of \([Ca^{2+}]\) 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 \([Ca^{2+}]\) 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 \([Ca^{2+}]_o\). This effect, however, is due to a greater sensitivity of unloaded shortening velocity to extracellular \(Ca^{2+}\), presumably as a result of larger \(Ca^{2+}\) influx into the cardiac cell and hence larger \(Ca^{2+}\) 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

We thank Dr. Ray Litten, Dr. Janice Parente, and Ms. Tin Nguyen for assistance with the isoenzyme determination.

References

26. Siemankowski RF, Wiseman MO, White HD: ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. Proc Natl Acad Sci USA 1985;82:658–662

KEY WORDS • isoproterenol • force–sarcomere velocity relation • hypothyroidism • isomyosin
Lack of effect of isoproterenol on unloaded velocity of sarcomere shortening in rat cardiac trabeculae.

P P de Tombe and H E ter Keurs

doi: 10.1161/01.RES.68.2.382

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

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/68/2/382