DITPA prevents the blunted contraction-frequency relationship in myocytes from infarcted hearts

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Litwin, Sheldon E., Dongfang Zhang, Phyllis Roberge, and Gregory D. Pennock. DITPA prevents the blunted contraction-frequency relationship in myocytes from infarcted hearts. Am. J. Physiol. Heart Circ. Physiol. 278: H862–H870, 2000.—Loss of the positive force-frequency relationship is a characteristic finding in failing hearts. The mechanisms of this change are not well understood. Myocardial infarction (MI) was induced in rabbits to produce left ventricular (LV) dysfunction. Beginning 1 day after MI, a subgroup of rabbits received diiodothyropropionic acid (DITPA) (3.75 mg·kg−1·day−1·sc) for 3 wk. We measured contractions, Ca2+ transients, action potentials, and sarcoplasmic reticulum (SR) Ca2+ content at different stimulation rates in single LV myocytes. The shortening-frequency relationship was markedly flattened in MI myocytes compared with control myocytes. In addition, Ca2+ transients, action potentials, and contractions were prolonged. Myocytes from DITPA-treated MI rabbits had preserved inotropic responses to increased stimulation rate and normal duration of action potentials and Ca2+ transients. SR Ca2+ content increased significantly when stimulation rate was increased from 0.5 to 2.0 Hz in control myocytes but did not change significantly in MI myocytes. Myocytes from DITPA-treated MI rabbits had a greater frequency-dependent increase in SR Ca2+ content compared with the untreated MI rabbits. Thus single myocytes from infarcted rabbit hearts have frequency-dependent abnormalities of contractility, Ca2+ cycling, and action potential repolarization. The flattened contraction-frequency relationship can be partially explained by an attenuation of the normal enhancement of SR Ca2+ content that occurs when stimulation rate is increased. Chronic DITPA administration after MI largely prevents the development of these abnormalities.

Ca2+ uptake and augmented Ca2+ release from the sarcoplasmic reticulum (SR) (6). By inference, impaired SR function in heart failure may contribute to the loss of frequency potentiation (16). Therefore, enhancing SR function might be expected to improve force-frequency relationships in heart failure. Unfortunately, treatments that act via cAMP-dependent pathways (i.e., phosphodiesterase inhibitors) increase mortality in patients with heart failure (30). Nonetheless, treatments that could improve SR function without increasing cAMP concentrations are theoretically attractive.

Thyroid hormone (T4) therapies have been proposed for the treatment of heart failure because T4 induces expression of sarco(end)oplasmic reticulum Ca2+ ATPase (SERCA2a) and the ryanodine receptor, and decreases expression of phospholamban (2). Because of the potential adverse effects of chronic treatment with T4 (facyardia and metabolic stimulation), there has been interest in developing T4 analogs that could improve cardiac performance without the other undesired effects. Diiodothyropropionic acid (DITPA) is a T4 analog that produces increases in peak first derivative of pressure with respect to time over a wide dosing range, but with only half of the chronotropic effect and the general metabolic stimulation of T4 (26). DITPA treatment lowers left ventricular (LV) end-diastolic pressure and improves resting and stressed cardiac output in rats and rabbits with postinfarction LV dysfunction (21, 27).

Thyroid hormone treatment enhances the in vivo force-frequency and relaxation-frequency relationships in normal primates (19). DITPA treatment produces similar effects in normal primates, except that chronic DITPA treatment is not associated with shifts in myosin isoforms or with an increase in resting heart rate (17). The effects of T4 or DITPA on force-frequency relationships in the failing heart have not previously been investigated. The goal of these studies was to test the hypothesis that DITPA treatment would prevent the abnormal force-frequency relationship in the diseased heart. In these studies, we employed a model of LV dysfunction due to myocardial infarction (MI) in the rabbit. This model has the advantages of strong clinical relevance (12) and a higher degree of similarity to human myocyte physiology than is seen in smaller rodents. In particular, the action potential morphology, myosin isoform profile, and relative dependence on the SR

IN NORMAL HEARTS, contractility is enhanced as stimulation rate increases. Loss of the positive force-frequency relationship has been reported in both human tissue and animal models of heart failure (3, 11, 20, 22). The normal enhancement of contractility that occurs with increases in stimulation rate is due to both increased myocardial infarction; calcium; ion channels; congestive heart failure; sarcoplasmic reticulum; rabbit; contractility; thyroid hormone; diiodothyropropionic acid

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as a source of activator Ca$^{2+}$, and force-frequency relationships in rabbit myocytes more closely resemble those in human myocytes than do those in the rat or the mouse (14).

**METHODS**

Male New Zealand White rabbits weighing 2.5–3.5 kg were used for all experiments. Animals were cared for according to the guidelines of the American Physiological Society. The studies were approved by the institutional animal care committees.

Production of myocardial infarction. MI was produced by ligating the circumflex artery as described previously (20, 21). In a minority of rabbits the circumflex artery is very small or intramyocardial in location and cannot be ligated. Two such rabbits were included along with twelve unoperated rabbits in the control group. Rabbits with MI were randomly assigned to DITPA (Sigma) or no treatment beginning on the first postoperative day. DITPA was administered at a dose of 3.75 mg·kg$^{-1}$·day$^{-1}$ for a total of 21 days. This dose has previously been shown to improve LV hemodynamics with little increase in heart rate (21, 27). Untreated rabbits were studied 3 wk after surgery.

Echocardiographic Doppler studies. Rabbits were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) intramuscularly, placed in the left lateral decubitus position, and imaged with a 7.5-MHz mechanical transducer (Sonos 100, Hewlett-Packard) (29). LV outflow tract (LVOT) diameter was measured in a parasternal long-axis view. A midventricular short-axis view was obtained, and the M-mode cursor crossed the anterior wall and bisected the papillary muscles. LV cavity dimensions were measured from the M-mode tracing using the leading edge method. Fractional shortening was calculated as

$$\text{FS} = \frac{LVID_d - LVID_s}{LVID_d}$$

where LVID$$_d$$ is LV internal diastolic dimension and LVID$$_s$$ is LV internal systolic dimension. In an apical long-axis view, pulsed wave Doppler recordings were made with the sample volume placed in the LVOT. Cardiac output (ml/min) was calculated as

$$\text{CO} = \pi \times (\text{LVOT diameter}/2)^2 \times \text{LVOT VTI} \times \text{HR}$$

where VTI is the velocity time integral (cm) and HR is heart rate (beats/min).

Myocyte isolation. Rabbits were killed by injection of Beuthanasia (0.5 ml, Schering-Plow Animal Health). The heart was excised and perfused with a Ca$$^{2+}$$-free, HEPES-buffered Tyrode solution (37°C) followed by a solution containing 0.1 mmol/l Ca$$^{2+}$$, 0.1% collagenase (Clu II, Worthington), and 0.01% protease (type XI; Sigma). After 15 min, the chambers were separated and weighed. The left ventricle was trimmed so that a 2- to 3-mm rim of surviving tissue remained around the clearly demarcated scar (20). This rim of tissue was then dissected free from the scar, minced, and strained. Tissue from the same region of the heart was used in the control rabbits and the infarcted rabbits. Myocytes from the region adjacent to the scar were used preferentially because previous work shows that there is greater cellular hypertrophy in this region (25). The yield of viable myocytes was 40–50% in all groups of rabbits. Only cells with rectangular shapes, clear striations, and absence of spontaneous contractions or membrane blebs were studied.

General features of single myocyte studies. Studies were performed within 8 h after cell dissociation. The cells were affixed with laminin to a glass coverslip that formed the bottom of a bath and then perfused with Tyrode solution (concentration in mmol/l: 138 NaCl, 1.0 MgCl$$_2$$, 4.4 KCl, 11.0 dextrose, 2.7 CaCl$$_2$$, and 12.0 HEPES, pH adjusted to 7.4 with NaOH; 30°C). This temperature was used to minimize rundown of currents and to optimize longevity of the cells. Previous works suggest that contraction-frequency relationships are not substantially different at 32°C or 37°C (11). Cells were viewed with an inverted microscope (Nikon Diaphot 200 or Olympus IX50). Cell motion was recorded using a video edge detection system (Crescent Electronics). Solutions superfusing individual cells were rapidly changed using a modification of a previously described solution switcher (35). The modified switcher utilized a single piece of double-barreled square glass tubing that was pulled to a fine tip over a flame. Whole cell patch-clamp studies were performed using borosilicate microelectrodes (Corning 7052; resistance 1–2 M$$\Omega$$). Current and cell motion were digitized at a sampling frequency of 1 ms (Digidata 1200, Axon Instruments) and stored on a personal computer for later analysis using pCLAMP 6 software (Axon Instruments). The low-pass Bessel filter on the Axopatch amplifier was set at 5 kHz for voltage-clamp experiments. During measurement of action potentials, membrane potential was sampled every 0.5 ms and the band-pass filter on the Axoclamp 2B amplifier was set at 3 kHz. No detectable aliasing occurred under these conditions.

Shortening-interval relationships in single myocytes. Frequency-dependent effects on contractility were measured in field-stimulated myocytes. This approach was used so that intracellular ionic composition and signaling processes were not disturbed and could change with stimulation rate. Five-millisecond pulses were applied with a pair of parallel platinum wires. Each cell was initially stimulated at 0.33 Hz for at least 1 min. Stimulation rate was successively increased from 0.33 to 0.5, 1.0, 2.0, and 3.0 Hz. Four consecutive steady-state contractions were recorded at each rate.

Measurement of action potentials. Action potentials were measured using the bridge mode of the voltage-clamp circuit. The Tyrode solution contained 1 mmol/l CaCl$$_2$$ and the pipette solution contained (concentration in mmol/l) 113 KCl, 5.5 dextrose, 5.0 K$_2$ATP, 10 HEPES, 0.02 EGTA, 0.5 MgCl$$$_2$$, and 10 NaCl, with pH adjusted to 7.1 using KOH. Five-millisecond current injections were adjusted to a level 10% above threshold. Cells were stimulated at 0.33 Hz for at least 1 min, and then action potentials were measured at 0.33, 0.5, 1.0, 2.0, and 3.0 Hz. Resting membrane potential, peak potential, and time to 20%, 50%, and 90% repolarization (APD$$_{20}$$, APD$$_{50}$$, and APD$$_{90}$$) were measured from 3–4 consecutive action potentials.

SR Ca$$^{2+}$$ content at different stimulation rates. SR Ca$$^{2+}$$ content was measured based on published methods (36). The pipette solution contained (concentration in mmol/l) 130 CsCl, 5.5 dextrose, 5.0 K$_2$ATP, 10 HEPES, 0.02 EGTA, 0.5 MgCl$$$_2$$, and 10 NaCl, with pH adjusted to 7.1 using CsOH. Before each recording, a 10-s train of conditioning pulses (300 ms) from −80 mV to −10 mV was applied. Steady-state SR Ca$$^{2+}$$ loading during the train was inferred because the amplitude of cell shortening increased over the first few beats and reached a plateau within 4–5 beats. During each train, the conditioning pulses were applied at frequencies of 0.5, 1.0, or 2.0 Hz. All three frequencies were used sequentially in each cell. These frequencies were chosen because during field stimulation the changes in contractile amplitude were largest...
Table 1. Cardiac chamber weights in control, MI, and DITPA-treated MI rabbits

<table>
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<tr>
<th></th>
<th>Control</th>
<th>MI</th>
<th>DITPA-treated MI</th>
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<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Atrial weight/BW, g/kg</td>
<td>0.53 ± 0.04</td>
<td>0.81 ± 0.06*</td>
<td>0.80 ± 0.04*</td>
</tr>
<tr>
<td>RV weight/BW, g/kg</td>
<td>0.93 ± 0.05</td>
<td>1.18 ± 0.08*</td>
<td>1.16 ± 0.05*</td>
</tr>
<tr>
<td>LV weight/BV, g/kg</td>
<td>2.61 ± 0.10</td>
<td>2.83 ± 0.09</td>
<td>2.80 ± 0.10</td>
</tr>
<tr>
<td>Scar weight, g</td>
<td>NA</td>
<td>1.82 ± 0.10</td>
<td>1.53 ± 0.06</td>
</tr>
<tr>
<td>Scar/LV</td>
<td>NA</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.01</td>
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Data are means ± SE; n, number of rabbits. Chamber weights (obtained after perfusion with collagenase) are expressed relative to body weight (BW). Diodothyropropionic acid (DITPA) or no treatment was administered for 3 wk beginning the first day after myocardial infarction (MI) (see Methods). Chamber weights and hemodynamic data were only obtained in 11/14 control rabbits. LV, left ventricular; RV, right ventricular; NA, not applicable. *P < 0.05 vs. control.

Table 2. Echocardiographic measurements in control, untreated MI and DITPA-treated MI rabbits

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MI</th>
<th>DITPA-treated MI</th>
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<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>149 ± 8</td>
<td>143 ± 5</td>
<td>148 ± 8</td>
</tr>
<tr>
<td>LVIDd, cm</td>
<td>1.71 ± 0.04</td>
<td>2.06 ± 0.05*</td>
<td>1.84 ± 0.05†</td>
</tr>
<tr>
<td>LVIDs, cm</td>
<td>1.11 ± 0.05</td>
<td>1.50 ± 0.04*</td>
<td>1.20 ± 0.06†</td>
</tr>
<tr>
<td>FS, %</td>
<td>35 ± 2.2</td>
<td>27 ± 1.3*</td>
<td>35 ± 2.0†</td>
</tr>
<tr>
<td>CI, ml·min⁻¹·kg⁻¹</td>
<td>86 ± 4</td>
<td>61 ± 3*</td>
<td>84 ± 4†</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, number of rabbits. HR, heart rate; LVIDd, LV internal diastolic dimension; LVIDs, LV internal systolic dimension; FS, fractional shortening; CI, cardiac index. *P < 0.05 vs. control. †P < 0.05 DITPA-treated MI vs. MI.
The number of cells used for each protocol are shown in the figures. Infarct size was not different in the untreated and DITPA-treated groups (Table 1). Rabbits with untreated MI showed evidence of chronic LV dysfunction, including increased atrial and right ventricular weights (Table 1). Echocardiographic measurements revealed significant LV dilatation, systolic dysfunction, and decreased cardiac output in the untreated MI rabbits (Table 2). DITPA treatment did not affect chamber weights but attenuated the LV dilatation and systolic dysfunction that were seen in the untreated MI rabbits (Tables 1 and 2).

Single myocyte shortening-frequency relationships. Compared with control myocytes, contractions in MI myocytes were smaller in magnitude, had decreased rates of shortening and relengthening, and had markedly prolonged time to peak shortening (Figs. 1 and 2). Increasing stimulation frequency was associated with significant increases in contractility in control myocytes (Figs. 1 and 2). However, there was little increase in contractility when pacing rate was increased in the MI myocytes. At a stimulation rate of 2.0 Hz, the untreated MI myocytes did not relax fully before the next contraction (Fig. 1). In contrast, all of the control and DITPA-treated MI myocytes were able to fully relax at a stimulation rate of 2.0 Hz. None of the myocytes from the untreated MI rabbits contracted regularly during every stimulus at 3.0 Hz (i.e., alternans developed). Thirteen of 42 control (31%), and 15 of 26 DITPA-treated MI myocytes (58%) contracted fully during each stimulus at 3.0 Hz. DITPA treatment almost completely prevented the frequency-dependent abnormalities of contractility and
relaxation in MI myocytes (P = not significant vs. control).

Action potential duration and stimulation frequency. Resting membrane potential was similar in myocytes from all three groups (Figs. 3 and 4A). The peak of the action potential was slightly but significantly more positive in the MI myocytes compared with control or DITPA-treated MI myocytes (Fig. 4B). At 0.33–2.0 Hz, action potential duration was significantly prolonged in myocytes from infarcted hearts compared with control myocytes (Figs. 3 and 4). The early portion of the plateau (APD20) was only slightly prolonged, whereas the later portions (APD50 and APD90) were markedly prolonged (Fig. 4C–4E). When stimulation rate was increased, action potential duration shortened in both control and MI myocytes. At a rate of 3.0 Hz, APD90 was not different in control and MI myocytes. In myocytes from DITPA-treated MI rabbits, action potential configuration was not different from controls at all stimulation frequencies (Figs. 3 and 4).

SR Ca\(^{2+}\) content and stimulation frequency. Examples of SR Ca\(^{2+}\) content measurements at different stimulation frequencies are shown in Fig. 5, and summary data are shown in Fig. 6. We found that releasable SR Ca\(^{2+}\) stores were increased in myocytes from the infarcted hearts at the slowest stimulation rate (0.5 Hz; Fig. 6A). When stimulation rate was increased, SR content increased significantly in control myocytes (slope = 0.36 ± 0.05) but not in untreated MI myocytes (slope = 0.09 ± 0.07; Fig. 6B). Myocytes from DITPA-treated MI rabbits showed an intermediate frequency-dependent increase in SR Ca\(^{2+}\) content (slope = 0.18 ± 0.04; Fig. 6B).

Intracellular Ca\(^{2+}\) transients. Compared with control myocytes, MI myocytes had a lower peak [Ca\(^{2+}\)], prolonged time to peak [Ca\(^{2+}\)], and slower decline in [Ca\(^{2+}\)] (Fig. 7). The amplitude and time course of Ca\(^{2+}\) transients were not different between DITPA-treated MI and control myocytes.
A flattened force-frequency relationship has been widely described in both human and experimental forms of heart failure (3, 11, 16, 20, 22) with a few exceptions (1). Whereas the exact causes of the blunted contraction-frequency relationship are still unclear, levels of SERCA2a protein have been reported to correlate with rate-dependent changes in contractile force (16). Interestingly, the force-frequency relationship in myocardium from failing human hearts can be improved acutely by administration of forskolin (23). Mulieri et al. (23) hypothesized that the forskolin-induced enhancement of the force-frequency relationship was attributable to an increase in intracellular cAMP levels. Presumably the increase in cAMP would promote SR Ca\(^{2+}\) uptake due to phosphorylation of phospholamban. Although these data are promising, chronic treatment of heart failure patients with agents that act primarily by increasing cAMP has been disappointing (30). Furthermore, cAMP-enhancing drugs may significantly reduce the economy of excitation-contraction coupling and the overall economy of contraction in failing human myocardium (15). The concept of improving SR function in the treatment of heart failure, however, may still be valid, and agents that improve SR function by alternate mechanisms may prove clinically useful.

We found that DITPA significantly improved the single myocyte shortening-frequency relationship in infarcted hearts. The time course of intracellular Ca\(^{2+}\) transients were similarly enhanced by DITPA treatment. These effects could be explained by a DITPA-mediated increase in the expression of SERCA2a relative to phospholamban. Both of these proteins are believed to be under transcriptional regulation by T\(_4\) (2, 8, 31). Preliminary data suggest that DITPA may also affect the expression of sarcolemmal and SR proteins important for excitation-contraction coupling (28). Finally, DITPA might indirectly affect Ca\(^{2+}\) cycling through signaling pathways that modulate excitation-contraction coupling proteins (e.g., Ca\(^{2+}\)/calmodulin-dependent kinase).

At stimulation rates of 0.33–2.0 Hz, action potential duration was significantly prolonged in the MI but not in the DITPA-treated MI myocytes. Action potential duration is determined largely by the balance of the depolarizing L-type Ca\(^{2+}\) current and the delayed rectifier K\(^{+}\) currents, rapidly activating (I\(_{K1}\)) and slowly activating (I\(_{Ks}\)) (18). Other repolarizing K\(^{+}\) currents [i.e., transient outward current (I\(_{to}\)) and inward rectifier K\(^{+}\) current (I\(_{K1}\))] may also contribute to phase 3 repolarization, although they are probably less important than I\(_{Ks}\). Several prior studies suggest that decreased expression and density of several of these K\(^{+}\) currents may underlie the action potential prolongation that is characteristic in hypertrophy or heart failure (24). In contrast, hyperthyroidism is believed to cause shortening of action potential duration (7). This may occur because of both acute effects on K\(^{+}\) currents (32) and chronic effects on K\(^{+}\) channel expression (34, 37). Our finding that DITPA restored a normal action potential duration in MI myocytes suggests that DITPA
may have prevented some of the abnormalities of K⁺ channel expression or function commonly seen in cardiac overload.

The effects of DITPA on action potential duration may be very important for the process of excitation-contraction coupling. Prolongation of action potentials may affect sarcolemmal Ca²⁺ fluxes via L-type Ca²⁺ channels and the Na⁺/Ca²⁺ exchanger (9). Action potential morphology affects both the electrical driving forces on Ca²⁺ and the extent of voltage-dependent inactivation of Ca²⁺ channels. Under steady-state conditions, prolongation of action potential duration has been reported to increase SR Ca²⁺ content but decrease the peak Ca²⁺ current and the peak rate of rise of the intracellular Ca²⁺ transient (9). These data are consistent with our observation that SR Ca²⁺ content tended to be enhanced in the MI myocytes at the slower stimulation rates, even though peak cellular shortening rate was decreased (Figs. 2 and 6). Because of the beneficial effects on action potential duration, it is

![Graph A](image1)

**Fig. 6.** Changes in SR Ca²⁺ content in single LV myocytes following stimulation at 0.5, 1.0, or 2.0 Hz (control, n = 28 myocytes from 6 rabbits; untreated MI, n = 28 myocytes from 3 rabbits; DITPA-treated MI, n = 30 myocytes from 4 rabbits). A: absolute values of calculated SR content (integral of caffeine-induced inward current/membrane capacitance; see METHODS). B: change in SR Ca²⁺ content (expressed relative to baseline at 0.5 Hz). Slopes of relative SR content vs. stimulation rate curves were calculated by linear regression (control = 0.36 ± 0.05; MI = 0.09 ± 0.05; DITPA-treated MI = 0.18 ± 0.04). Membrane capacitance for each group is as follows: control = 143 ± 7; MI = 143 ± 9; DITPA-treated MI = 135 ± 6 pF). Data are means ± SE. *P < 0.05 vs. control for each frequency. †P < 0.05, MI vs. control for the slope of the relationship. ‡P < 0.05, slope of relationship for DITPA-treated MI vs. untreated MI. NS, not significant.

![Graph B](image2)

**Fig. 7.** Ca²⁺ transients in field-stimulated myocytes. A: representative examples of Ca²⁺ transients from each group. Peak amplitude is lower, and time to peak and time to 50% decline in Ca²⁺ are prolonged in time from peak to 50% decline in [Ca²⁺]i (T₁/₂). MI myocyte but not in are DITPA-treated MI myocyte. Summary data are from control (n = 35 myocytes from 3 rabbits), MI (n = 42 myocytes from 5 rabbits), and DITPA-treated MI rabbits (n = 26 myocytes from 2 rabbits). F, fluorescence intensity; F₀, resting fluorescence intensity. B: peak intracellular Ca²⁺ concentration ([Ca²⁺]i). C: time to peak [Ca²⁺]i. D: time from peak to 50% decline in [Ca²⁺]i (T₁/₂). *P < 0.05 vs. control. †P < 0.05, DITPA-treated MI vs. MI.
conceivable that DITPA or similar compounds could prove to be useful inotropic agents with a smaller risk of provoking arrhythmias or sudden death. DITPA might be particularly useful for bradycardia- or pause-dependent arrhythmias, because the effects of DITPA on action potential duration appear to be more pronounced at slower stimulation rates.

Myocytes from infarcted hearts did not show increases in SR Ca$^{2+}$ content when stimulation rate was increased. Although the use of caffeine may have limitations in determining the absolute quantity of Ca$^{2+}$ in the SR, the relative changes for each cell should be reliable. Another consideration in the interpretation of these experiments is that the Na$^+$/Ca$^{2+}$ exchanger current density is increased in MI myocytes (20). Such a change should increase the peak amplitude of the caffeine-induced inward current for a given SR release, but the current integral (the total amount of Ca$^{2+}$ extruded) should not change (38).

The main factors producing normal rate-dependent enhancement of SR Ca$^{2+}$ content include increased time-averaged sarcolemmal Ca$^{2+}$ entry via L-type channels and enhancement of SR Ca$^{2+}$ uptake due to phosphorylation of phospholamban (5). It is possible that the blunted frequency staircase in diseased hearts reflects abnormalities of one of these mechanisms. However, it is also possible that the failure to increase SR Ca$^{2+}$ content in the postinfarction myocytes occurs because the SR is already “full,” even at slower stimulation rates. Such a situation could conceivably result if the fractional SR Ca$^{2+}$ release were diminished. In normal myocytes, the fractional SR release has been shown to increase as the total SR Ca$^{2+}$ content increases (4). Inability to increase the fractional SR Ca$^{2+}$ release might result from impaired coupling of sarcolemmal Ca$^{2+}$ entry and SR Ca$^{2+}$ release. This hypothesis has a precedent because some investigators have shown evidence of defective triggering of SR Ca$^{2+}$ release in myocytes from failing hearts (13). Although we did not measure the “gain” of SR Ca$^{2+}$ release in these studies, our data are compatible with an alteration in the sensitivity of the Ca$^{2+}$ release apparatus.

Our data expand on previous work with thyroid hormone (10) by demonstrating that the $T_3$ analog DITPA restores frequency potentiation in single myocytes from a postinfarction model. Compared with $T_3$, DITPA has the clear advantage of not causing tachycardia. The current work extends our knowledge about the mechanism(s) of action of thyroid hormone analogs by demonstrating for the first time that DITPA prevents frequency-dependent abnormalities of SR function in intact myocytes. Preliminary studies suggest that DITPA enhances the expression of SR proteins involved in Ca$^{2+}$ cycling (28).

Limitations. There is potential for significant selection bias or sampling error introduced by studying single cells isolated from infarcted hearts. By studying a fairly large number of cells (20–30 per group, per protocol) with only 5–10 cells coming from any one rabbit, sampling errors should have been minimized. Even so, it is possible that the “sickest” cells might not survive the isolation process. We do not think this is the case because cells isolated from the infarcted rabbit heart have the expected morphological and electrophysiological characteristics of hypertrophied or failing hearts (i.e., myocyte hypertrophy, action potential prolongation, and abnormalities of contraction and relaxation). It is possible, although we think it is unlikely, that the effects we attribute to DITPA were the indirect result of daily injections rather than a direct effect of the agent. We cannot rule out this possibility because we did not give vehicle injections to the untreated MI or control rabbits. Another issue is that the effects of DITPA are complex and almost certainly multifactorial in etiology. Despite some uncertainty about the underlying mechanisms, the demonstration of benefit at the level of the single myocyte is clear and there is substantial indirect evidence that SR function was improved following treatment.

In conclusion, these results show for the first time that DITPA treatment prevents prolongation of action potential duration and the development of abnormal contractile responses to increasing stimulation frequency in myocytes from infarcted hearts. Our data are consistent with a DITPA-mediated enhancement of SR function, although the exact mechanism of action remains to be determined. This study demonstrates how DITPA exerts direct effects on the myocyte and furthers our understanding of thyroid hormone analogs in the treatment of heart failure.

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