Combination Treatment With Captopril and the Thyroid Hormone Analogue 3,5-Diiodothyropropionic Acid

A New Approach to Improving Left Ventricular Performance in Heart Failure

Gregory D. Pennock, MD; Thomas E. Raya, MD; Joseph J. Bahl, PhD; Steven Goldman, MD; Eugene Morkin, MD

Background. An agent that improves left ventricular (LV) performance by non-cAMP-mediated mechanisms would be valuable in the treatment of chronic heart failure. We have shown earlier that the thyroid hormone analogue 3,5-diiodothyropropionic acid (DITPA) binds to nuclear receptors, alters transcription of T3-responsive genes, and increases +dP/dt max in hypothyroid rats with substantially less effect on heart rate and metabolism than thyroid hormone, which makes it a selective cardiotonic agent.

Methods and Results. To determine whether DITPA might be useful in treating heart failure, we compared chronic treatment with normal saline, captopril (2 g/L), or the combination of DITPA (375 μg/100 g) and captopril (2 g/L) in Sprague-Dawley rats beginning 3 weeks after coronary artery ligation. Both DITPA/captopril and captopril treatment decreased LV end-diastolic pressure compared with controls (21±2 and 26±2 mm Hg, respectively, vs 34±3 mm Hg, P<.05 for each). The addition of DITPA to captopril produced a 36% increase in resting cardiac index (P<.05) and shifted the cardiac function curve upward and to the left, indicative of enhanced myocardial performance. Also, DITPA/captopril compared with captopril treatment or control produced an increase in the rate of LV relaxation, as manifested by a decrease in τ, the time constant of LV pressure decline (17.5±1.0 vs 22.2±1.7 milliseconds, P<.05) and a larger absolute value for −dP/dt max (−4561±361 vs −3346±232 mm Hg/s, P<.05). These changes occurred without changes in heart rate, LV mass, LV systolic pressure, or peripheral resistance relative to captopril treatment (P>.05).

Conclusions. The combination of DITPA and captopril improved cardiac output, increased −dP/dt max, and increased the rate of LV relaxation to a greater extent than captopril treatment in the rat postinfarction model of heart failure. Use of a cardiotonic analogue of thyroid hormone represents a new approach to improving LV performance and may be a useful adjunct to afterload reduction for the treatment of heart failure. (Circulation. 1993;88:1289-1298.)

KEY WORDS • heart failure • cardiotonic agents • thyroid

Several recent clinical trials have documented that vasodilators, especially angiotensin-converting enzyme (ACE) inhibitors, improve left ventricular (LV) function and increase survival in patients with chronic heart failure.1-2 Despite ACE inhibitors, however, many patients continue to have decreased exercise capacity and symptoms of heart failure. For those with New York Heart Association functional class IV symptoms, a recent study found a yearly mortality of 36%.3 For these reasons, there continues to be interest in the development of agents that may improve LV performance and prolong survival.

Earlier studies have suggested that there are abnormalities of intracellular cyclic AMP (cAMP) formation in the failing heart.4 Consequently, the development of inotropic drugs has focused on agents that increase intracellular cAMP, either by direct stimulation of adenylate cyclase activity or inhibition of phosphodiesterase activity.5,6 Although phosphodiesterase inhibitors improve symptoms, exercise capacity, and hemodynamics, development of tolerance has limited the long-term use of these agents.7-9 In addition, two recent multicenter, randomized, placebo-controlled trials with milrinone and enoximone have found increased mortality in the treatment groups compared with placebo.9,10 It is not known whether all inotropic agents will increase mortality in patients with LV dysfunction. The results of vasodilator trials suggest that considerable heterogeneity exists among various drugs in terms of their effects on hemodynamics and survival. It is possible that drugs with inotropic actions mediated by alternative, non-cAMP-mediated pathways may improve both LV hemodynamics and survival.

Thyroid hormone improves cardiac output by a combination of effects on myocardial contractile perfor-
mance and the peripheral circulation. Cardiac effects of thyroid hormone include increases in cardiac index, heart rate, and +dP/dt and improvement in LV relaxation. In addition, peripheral resistance is decreased, and changes in the venous circulation occur that promote venous return. Enhancement of myocardial contractility and relaxation by thyroid hormone and thyroid hormone analogues in papillary muscle preparations has been shown to be independent of loading conditions. These effects on myocardial performance are thought to be initiated through binding to high-affinity nuclear receptors, which are now believed to be products of the c-erb A protooncogenes. Binding to these receptors results in coordinated changes in the expression of a network of thyroid hormone-responsive genes. Among the genes affected are those encoding contractile proteins, proteins associated with amino acid and ion transport, and enzymes involved in energy metabolism. The sum of these transcriptional changes is thought to provide the molecular basis for enhancement of cardiac muscle function. Thus, thyroid hormone and analogues of thyroid hormone can be viewed as regulators of genes that alter cardiac performance at the most fundamental level.

Thyroid hormone itself is not useful as a pharmacological agent for the long-term treatment of heart failure, because the doses required to sustain effects on contractile performance, such as increases in the rate of pressure development (+dP/dt, max), are associated with tachycardia. Also, the decrease in LV end-diastolic pressure (EDP) observed after 72 hours of treatment is not sustained with longer administration (10 to 12 days). An analogue of thyroid hormone with low metabolic activity, 3,5-diiodothyropropionic acid (DITPA), recently has been shown to have relatively selective inotropic activity in hypothyroid rats. DITPA differs chemically from thyroxine (T4) in the absence of iodides at the 3',5' positions and in the substitution of a propionic acid side chain for the alanine side chain (Fig 1). DITPA binds to bacterially expressed thyroid hormone nuclear receptors, induces α-myosin heavy chain mRNA expression in cultured cardiac myocytes, and produces positive inotropic activity in hypothyroid rats with less effect on heart rate than T4. With doses of T4 and DITPA that produced equivalent increases in +dP/dt, max, the effects of DITPA on heart rate and hepatic α-glycerol phosphate dehydrogenase activity were about 50% less.

The purpose of the present study was to determine whether DITPA is useful for the treatment of heart failure. Rats with heart failure after ligation of the left anterior descending coronary artery were treated with captopril, the combination of DITPA and captopril, or saline (infarcted control rats) beginning 3 weeks after myocardial infarction. Earlier studies have established the value of the rat postinfarction model in the study of heart failure. The specific question addressed was, "Does the combination of DITPA and captopril provide hemodynamic improvement over treatment with captopril alone?" At the end of treatment, several parameters of LV performance were measured, including LV pressures, cardiac output before and after volume stress, and ventricular diastolic pressure-volume relations. The results indicate that both drug regimens lowered LV EDP compared with the saline-treated control group. More importantly, the combination of DITPA and captopril improved cardiac output, increased the rate of LV relaxation, and shortened the time constant of isovolumic LV relaxation to a greater degree than captopril.

Methods

The animals used in this study were handled according to the animal welfare regulations of the University of Arizona and the Tucson Veterans Administration Hospital, and the protocol was approved by the animal use committees of these institutions. These regulations are in accordance with principles of animal use of the American Physiological Society and the American Heart Association.

Experimental Infarction

Myocardial infarction was produced in adult male Sprague-Dawley rats (175 to 275 g) with techniques similar to those described earlier. In brief, the rats were anesthetized with methoxyflurane, and a left thoracotomy was performed. The heart was expressed from the thorax, and a ligature was placed around the proximal left coronary artery. The heart was returned to the chest, and the thorax was closed. An immediate survival rate of ≈50% was achieved. The rats were maintained on standard rat chow (Ralston Purina Co, St Louis, Mo) with water ad libitum.

After 3 weeks, rats were anesthetized, and a nine-lead ECG with six limb leads and three chest leads was performed. Rats with evidence of large myocardial infarctions by criteria described by Pfeffer et al were selected for study. Briefly, the presence of Q waves >1 mV in the limb leads (I or AVL) and the sum of R waves in the precordial leads <10 mV were used as criteria for a large myocardial infarction. Myocardial infarct size was quantified postmortem in each animal by use of techniques described earlier.

Treatment Groups

Three weeks after infarction, age-matched rats with large infarctions by ECG criteria were randomly assigned to one of three treatment groups: (1) treatment...
with captopril for 21 days without DITPA, (2) treatment with captopril for 21 days and a daily subcutaneous dose of DITPA for the last 10 days of treatment, and (3) a control group treated with a daily dose of 0.9% saline for 10 days. The chosen dose of DITPA was based on a preliminary dose-ranging study in which animals received three incremental doses of DITPA (375 μg/100 g, 1000 μg/100 g, and 1500 μg/100 g) for 10 days. The dose selected provided the best ratio of +dP/dt max to heart rate (Fig 2). The 10-day treatment period for DITPA was selected because evidence of improved LV function and return of the pattern of myosin isoenzymes toward normal occurs by this time.22

Stock solutions of DITPA (Sigma Chemical Co, St Louis, Mo; 3.75 mg/mL) were prepared by dissolving the powder in 0.1N NaOH and diluting with 0.9% saline (pH 8 to 9). Daily doses of 375 μg/100 g were administered subcutaneously. A volume of 0.1 mL was injected for both DITPA and control (saline) treatments. Captopril (Bristol-Myers Squibb Co, Princeton, NJ) was administered in drinking water at a final concentration of 2 g/L.

**Hemodynamics**

**Baseline LV performance.** At the end of the treatment period, the rats were anesthetized with methoxyflurane, and a 1-mm micromanometer-tipped catheter (Millar Instruments, Houston, Tex) was inserted into the right carotid artery. The catheter was advanced into the aorta and then into the LV under constant pressure monitoring. The zero pressure baseline was obtained by placing the pressure sensor in 37°C saline before measurements. After initial recordings were obtained, the catheter was exteriorized to the midcervical region, and the animals were allowed to recover from anesthesia and surgery for at least 4 hours before hemodynamic measurements were obtained. Pressure data were recorded on a physiological recorder (model 2400, Gould Instrument Co, Cleveland, Ohio). The maximal rates of LV pressure development and decline (+dP/dt max and −dP/dt max, respectively) were obtained from a differentiating circuit in the recorder with the high-filter frequency cutoff set at 100 Hz. Three successive measurements 5 minutes apart were performed for each parameter to ensure reproducibility, and averaged data are reported.

**Stressed LV performance.** After completion of hemodynamic measurements, animals were anesthetized with thiobutabarbitral (Andrew Lockwood Associates, East Lansing, Mich). Approximately 40 minutes was allowed for complete anesthesia. The left femoral artery was then exposed, and a 0.64-mm thermistor microprobe (Columbus Instruments, Columbus, Ohio) was advanced to the descending aorta. The contralateral femoral artery and the superior vena cava were cannulated with PE 50 tubing for measurement of mean arterial pressure in the descending aorta and mean right atrial pressure, respectively. Baseline thermodilution cardiac outputs were obtained at core body temperatures between 37.5°C and 38°C with a Cardiotherm 500 (Columbus Instruments) instrument. Heart rate was obtained simultaneously for calculation of stroke volume. Blood was taken from a donor rat, and an amount equivalent to 10% total blood volume (6.2 mL/kg) was infused into the right atrium. LV EDPs and cardiac outputs were recorded before and after the volume infusion. Animals were intubated with a 20-gauge catheter and ventilated with a volume-cycled respirator (Harvard Apparatus, South Natick, Mass). A median sternotomy was performed, and a 2-0 silk tie was placed around the aorta. A piece of polyethylene tubing 2 to 3 cm long (1.75-mm ID) was placed over the tie and used as an occlusion device. The aorta was occluded for 2 to 3 seconds to produce isovolumic (except for coronary flow) contractions. The peak LV developed pressure was determined by measuring the difference in peak systolic pressures and EDPs after five stable beats.

**Isolated LV Pressure-Volume Relations**

After completion of all measurements, KCl was rapidly injected into the right atrial catheter to arrest the heart in diastole. Pressure-volume data were recorded by methods described earlier.12,28-30 Briefly, the heart was removed rapidly, and the right ventricle was incised. A double-lumen catheter attached to a pressure transducer (Statham 231d, Gould Instrument Co) and an infusion pump (Sage 341, Orion Research, Cambridge, Mass) was passed into the left ventricle and secured with an aortic ligature. The ativoventricular groove was identified, and a ligature was passed around the heart and tied to isolate the left atrium from the left ventricle. After gentle aspiration of the LV cavity to remove any residual blood and to reduce the pressure to −5 mm Hg, normal saline was infused at 1 mL/min into the suspended left ventricle while pressure was simultaneously recorded. Saline was infused until the pressure increased to 40 mm Hg. Three curves were obtained from

![Graph of dose-ranging experiments comparing heart rate and +dP/dt max in rats with large myocardial infarcts treated with 3,5-didodothyropropionic acid (DITPA) at dosages of 0, 375, 1000, and 1500 μg/100 g body weight for 10 days. Numbers below data points represent the dosage of DITPA (μg/100 g). HR, heart rate; bpm, beats per minute; +dP/dt max, maximal rate of left ventricular pressure development. n=3 rats for each dose. Values are mean±SEM.](attachment:image.png)
TABLE 1. Treatment Effects on Body Weights and Heart Weights for Infarcted Control, Captopril, and DITPA/Captopril-Treated Rats With Large Myocardial Infarctions

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>Captopril (n=10)</th>
<th>DITPA/Captopril (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>369±11</td>
<td>381±10</td>
<td>372±11</td>
</tr>
<tr>
<td>LV wt (g)</td>
<td>0.729±0.047</td>
<td>0.625±0.022</td>
<td>0.680±0.027</td>
</tr>
<tr>
<td>RV wt (g)</td>
<td>0.412±0.034</td>
<td>0.257±0.020*</td>
<td>0.276±0.026*</td>
</tr>
<tr>
<td>Heart wt (g)</td>
<td>1.141±0.030</td>
<td>0.883±0.019*</td>
<td>0.956±0.041*</td>
</tr>
<tr>
<td>LV/BW</td>
<td>1.98±0.13</td>
<td>1.70±0.07</td>
<td>1.83±0.08</td>
</tr>
<tr>
<td>RV/BW</td>
<td>1.13±0.11</td>
<td>0.69±0.06*</td>
<td>0.74±0.06*</td>
</tr>
<tr>
<td>HW/BW</td>
<td>3.12±0.11</td>
<td>2.33±0.06*</td>
<td>2.57±0.09*</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>46.4±3.1</td>
<td>45.4±1.6</td>
<td>43.6±3.3</td>
</tr>
</tbody>
</table>

*P<.05 vs infarcted controls.

Each ventricle within 10 minutes of the cardiac arrest, and the averaged values of volume indexed to body weight at given pressures were reported.

Calculations

**Time constant of LV relaxation.** The time constant of LV relaxation (τ) was measured by use of published techniques. In brief, LV pressure of 100 to 150 consecutive cardiac cycles was digitized with an IBM AT computer at a frequency of 1000 Hz. For each cardiac cycle, dP/dt max, −dP/dt max, peak systolic pressure and LV EDP were identified. The value of LV EDP was subtracted from each pressure point on the pressure decay curve of that cycle beginning at −dP/dt max and ending at a point equal to the EDP. The resulting values (P) were then fit to the relation:

\[ dP/dt = -a(P-P_b) \]

where \( P_b \) is the intercept of pressure at \( dP/dt = 0 \), a, the time constant for exponential pressure decay, is calculated by the least-squares method (r>.99), and \( r = -1/a \).

**Total peripheral resistance index.** Total peripheral resistance index was calculated as the difference in mean arterial pressure and right atrial pressure divided by the cardiac index.

**Ejection fraction index.** The ejection fraction index was calculated by dividing in vivo stroke volume by the baseline (before volume load) end-diastolic volume as determined from ex vivo pressure-volume relations.

**Chamber stiffness.** The overall chamber stiffness constant, \( K_c \), was determined from methods similar to those described earlier. Briefly, pairs of simultaneous pressure-volume points were recorded and fitted (r=.99) to the exponential equation:

\[ P = P_o e^{K_v} \]

where \( P_o \) is the pressure at zero ventricular volume and \( V \) is the ventricular volume index.

**LV volume-mass ratio.** Ventricular cavity volume at a distending pressure of 10 mm Hg was determined from the passive pressure-volume relation. LV wall volume (Vw) was determined from the mass of the left ventricle, such that Vw=LV mass (g)+1.06 (muscle density). This relation assumes that ventricular muscle is incompressible.

**Myocardial oxygen consumption.** Estimates of myocardial oxygen consumption (MVo2) were made from measurements of heart rate (HR), systolic pressure (Psys), and +dP/dt according to a regression equation proposed by Hutter et al:

\[ MVo2 = C_0 + C_1 \times HR \times P_{sys} + C_2 \times HR \times +dP/dt_{max} \]

where \( C_0 = 0.5355 \)
\( C_1 = 0.1282 \times 10^{-3} \) mm Hg \( ^{-1} \times \) min
\( C_2 = 0.2210 \times 10^{-3} \) mm Hg \( ^{-1} \times \) s \times \) min

Estimates made by this equation have been found to agree closely with measured values for MVo2 in the working rat heart under a wide range of hemodynamic conditions.

**Data Analysis**

Data are reported as mean±SEM. ANOVA was applied to compare mean values among groups. When ANOVA showed statistical significance by F test, intergroup comparisons were made by the Student-Newman-Keuls procedure. A value of \( P<.05 \) was considered statistically significant. Although equal numbers of animals were randomized to each group, differences occurred in the number of observations in each group.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>Captopril (n=8)</th>
<th>DITPA/Captopril (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>372±7</td>
<td>349±6</td>
<td>366±8</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>124±4</td>
<td>111±5</td>
<td>113±3</td>
</tr>
<tr>
<td>LV EDP (mm Hg)</td>
<td>34±3</td>
<td>26±2*</td>
<td>21±2*</td>
</tr>
<tr>
<td>+dP/dt max (mm Hg/s)</td>
<td>5691±382</td>
<td>5006±542</td>
<td>6214±487</td>
</tr>
<tr>
<td>−dP/dt max (mm Hg/s)</td>
<td>−3841±247</td>
<td>−3346±232</td>
<td>−4561±361+</td>
</tr>
<tr>
<td>τ (ms)</td>
<td>18.8±0.9</td>
<td>22.2±1.7</td>
<td>17.5±1.0+</td>
</tr>
<tr>
<td>MVo2 (µmol · min (^{-1} ) · g(^{-1} ))</td>
<td>11.11±0.51</td>
<td>9.35±0.61</td>
<td>11.18±0.76</td>
</tr>
</tbody>
</table>

DITPA, 3,5-diodothyropropionic acid; HR, heart rate; bpm, beats per minute; LVSP, left ventricular systolic pressure; LV EDP, left ventricular end-diastolic pressure; +dP/dt max, maximum rate of LV pressure development; −dP/dt max, maximum rate of LV pressure decline; τ, time constant of monoeponential LV pressure decay; MVo2, myocardial oxygen consumption. Values are mean±SEM.

*P<.05 vs infarcted controls; 1P<.05 vs captopril alone.
because of the mortality associated with instrumenting animals with heart failure. Either by chance or because of functional improvement, the DITPA/captopril group contained the largest number of data points.

Results

Each animal was inspected at the end of the treatment period and before instrumentation. No subjective differences were observed among the three experimental groups. In addition, body weights before and after treatments were not different among the groups (Table 1). The average infarct size was >40% for all groups, and there were no differences in infarct size among the groups (P>.05). Treatment with captopril and the combination of DITPA and captopril had no effects on body weight, LV weight, or LV-to-body weight ratio. In both the captopril and DITPA/captopril treatment groups, right ventricular and total (LV+right ventricular) heart weights were decreased compared with saline-treated controls (P<.05). Presumably, in the captopril- and DITPA/captopril-treated groups, there may have been less RV hypertrophy because of reductions in right heart pressures.

Hemodynamic responses to treatments are shown in Table 2. There were no differences in mean heart rates or in mean LV peak systolic pressures among the three groups (P>.05). Although the average maximal rate of pressure development (+dP/dt max) was highest for the DITPA/captopril group, this value did not achieve statistical significance. Values for heart rate, LV peak systolic pressure, and LV +dP/dt were used to estimate MVO₂ according to the regression equations given in “Methods” (Table 2). No significant differences among the three groups in calculated MVO₂ were observed (P>.05).

Analysis of LV pressure curves in conscious animals after instrumentation revealed that LV EDP was decreased significantly (P<.05) with both captopril treatment (~24%) and DITPA/captopril (~38%) vs saline-treated controls. There were no differences in average values for LV EDP between rats treated with captopril and those receiving DITPA/captopril (P>.05), however. The rate of LV relaxation, as measured by −dP/dt max and τ, was unchanged with captopril treatment compared with controls. The addition of DITPA to captopril increased the absolute value for the maximal rate of pressure decline (−dP/dt max) by 36%, on the average, compared with the captopril-treated group (P<.05). Furthermore, τ was 21% shorter with DITPA/captopril treatment than with captopril administration (P<.05).

The average baseline cardiac index was 36% higher in the DITPA/captopril group than in the captopril treatment group (P<.05) (Table 3). After a 10% blood volume load, the average cardiac index was increased in all three groups. In the group treated with DITPA/captopril, the average cardiac index was 28% higher than with captopril treatment (P<.05). Furthermore, as a result of the decrease in LV EDP and the increase in baseline and stressed cardiac indexes, the cardiac function curve for the DITPA/captopril group was shifted upward and to the left compared with both the captopril-treated group and saline-treated controls (Fig 3). This shift of the curve suggests that DITPA improved myocardial performance.

![Cardiac function curves before (closed symbols) and after (open symbols) 10% blood volume infusion. The effects of the volume load are compared in saline-treated infarcted controls (n=8) and rats treated for 10 days with captopril (n=8) and the combination of 3,5-diodothiopropionic acid (DITPA) and captopril (n=13) beginning 3 weeks after large myocardial infarctions. CI, cardiac index; LV EDP, left ventricular end-diastolic pressure. Values are mean±SEM. *P<.05 LV EDP vs infarcted saline controls; †P<.05 CI vs captopril treatment.](http://circ.ahajournals.org/doi/abs/10.1161/01.CIR.106.2.1293)

**TABLE 3. Cardiac Indexes, Stroke Volume Indexes, and Ejection Fractions Before and After Blood Volume Load for Infarcted Control, Captopril, and DITPA/Captopril-Treated Rats With Large Myocardial Infarctions**

<table>
<thead>
<tr>
<th></th>
<th>Before volume load</th>
<th>After volume load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=8)</td>
<td>Captopril (n=8)</td>
</tr>
<tr>
<td>CI (mL·min⁻¹·kg⁻¹)</td>
<td>280±15</td>
<td>227±19</td>
</tr>
<tr>
<td>SVI (mL/kg)</td>
<td>0.90±0.05</td>
<td>0.73±0.08</td>
</tr>
<tr>
<td>EFI (%)</td>
<td>0.39±0.05</td>
<td>0.33±0.05</td>
</tr>
<tr>
<td></td>
<td>Control (n=8)</td>
<td>Captopril (n=8)</td>
</tr>
<tr>
<td>CI (mL·min⁻¹·kg⁻¹)</td>
<td>319±23</td>
<td>288±26</td>
</tr>
<tr>
<td>SVI (mL/kg)</td>
<td>1.05±0.06</td>
<td>0.93±0.10</td>
</tr>
<tr>
<td>EFI (%)</td>
<td>0.44±0.07</td>
<td>0.41±0.06</td>
</tr>
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</table>

DITPA, 3,5-diodothiopropionic acid; CI, cardiac index; SVI, stroke volume index; EFI, ejection fraction index. Values are mean±SEM. *P<.05 vs before volume load captopril; †P<.05 vs after volume load captopril; ‡P<.05 vs before volume load DITPA/captopril.
TABLE 4. Mean Arterial Pressures, Right Atrial Pressures, Total Peripheral Resistance Index, and Peak Developed Pressures for Infarcted Control, Captopril, and DITPA/Captopril-Treated Rats With Large Myocardial Infarctions

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Captopril (n=8 to 10)</th>
<th>DITPA/captopril (n=10 to 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>97±7</td>
<td>87±4</td>
<td>84±5</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>2.2±0.7</td>
<td>1.3±0.3</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>TPRI (mm Hg·mL⁻¹·min⁻¹·kg⁻¹)</td>
<td>0.36±0.04</td>
<td>0.38±0.04</td>
<td>0.28±0.03</td>
</tr>
<tr>
<td>PDP (mm Hg)</td>
<td>126±10</td>
<td>125±8</td>
<td>140±11</td>
</tr>
</tbody>
</table>

DITPA, 3,5-diiodothyropropionic acid; MAP, mean arterial pressure; RAP, right atrial pressure; TPRI, total peripheral resistance index; PDP, peak developed pressure. Values are mean±SEM. No statistical differences were observed for any of the measurements.

The stroke volume index, ejection fraction index, and peak LV developed pressure were higher, and the total peripheral resistance index was lower, for the DITPA/captopril-treated group compared with the captopril and control groups. None of these changes reached statistical significance (P>.05), however. Also, there were no significant differences in mean right atrial pressure or mean arterial pressure among the three groups (Table 4).

The effects of DITPA on isolated diastolic pressure-volume relations of the LV are shown in Table 5 and Fig 4. The LV volume indexes were not statistically different among the three groups at any pressure ≤40 mm Hg. Furthermore, there were no differences among the groups in the volume mass ratios in Kₐ (P>.05). Although operating LV end-diastolic volume indexes were not different among the three groups (P>.05), the mean values for LV end-diastolic volume indexes were lower in the groups treated with captopril and DITPA/captopril. The pressure-volume relations were similar in all groups, but treatment with captopril and DITPA/captopril caused the hearts to operate on a lower (flatter) portion of the curve compared with the infarcted control group (Fig 5). LV EDP was greater for the infarcted control group at LV volumes measured during both baseline and volume-stressed conditions.

TABLE 5. LV Cavity-to-Wall Volume Ratios, LV Chamber Stiffness Constants, and LV Operating End-Diastolic Volumes for Infarcted Control, Captopril, and DITPA/Captopril-Treated Rats With Large Myocardial Infarctions

<table>
<thead>
<tr>
<th></th>
<th>Infarcted control (n=7)</th>
<th>Captopril (n=9)</th>
<th>DITPA/captopril (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/Vₑ</td>
<td>1.03±0.14</td>
<td>1.63±0.22</td>
<td>1.21±0.14</td>
</tr>
<tr>
<td>Kₑ</td>
<td>2.19±0.31</td>
<td>1.81±0.18</td>
<td>1.77±0.08</td>
</tr>
<tr>
<td>LV EDVI (mL/kg)</td>
<td>2.83±0.26</td>
<td>2.54±0.13</td>
<td>2.38±0.33</td>
</tr>
</tbody>
</table>

LV, left ventricular; DITPA, 3,5-diiodothyropropionic acid; V/Vₑ, ratio of ventricular cavity to wall volume; Kₑ, ventricular chamber stiffness constant; LV EDVI, LV end-diastolic volume index at the operating LV end-diastolic pressure. Values are mean±SEM. No statistical differences were observed for any of the measurements.

Discussion

We have demonstrated that the addition of the thyroid hormone analogue DITPA to captopril improves overall LV function compared with treatment with captopril alone in rats with heart failure after myocardial infarction. In particular, DITPA markedly increased the cardiac index at baseline and under conditions of volume stress. In addition, improvement in...
LV performance occurred without an increase in heart rate, which is consistent with the selective cardiotonic action of DITPA observed in hypothyroid rats. When DITPA was combined with captopril for the treatment of heart failure, the most desirable qualities of both DITPA (improvement in cardiac performance) and captopril (decreased preload and afterload) were evident.

Mechanisms of Increased Cardiac Output

Earlier studies have demonstrated that thyroid hormone increases cardiac output because of changes in both intrinsic myocardial properties and the peripheral circulation. Although the combination of DITPA and captopril did not increase resting cardiac index significantly above the value in infarcted controls, in the control group this value was achieved at the expense of markedly elevated LV EDP. With the combination of DITPA and captopril, baseline and stressed cardiac indexes were improved compared with captopril treatment. The LV EDP was decreased, and there were only minor changes in peripheral resistance. Thus, the upward and leftward shift of the cardiac function curve suggests that enhanced myocardial performance was partly responsible for the improvement in cardiac output.

Abnormalities in LV diastolic function are frequently present in the failing left ventricle and in some cases may supersede systolic dysfunction as the predominant cause of congestive symptoms. In the present study, improvement in diastolic function, as reflected in changes in \( \frac{-dP}{dt_{\text{max}}} \) and \( \tau \), may have contributed to the increase in cardiac index and lower LV EDP observed with DITPA treatment. Whereas captopril tended to prolong relaxation, the addition of DITPA facilitated (shortened) relaxation. Since no differences were observed with respect to ex vivo pressure-volume relations, the improvement in relaxation and cardiac index probably was not the result of changes in the passive elastic properties of the ventricle but rather of alterations in active, energy-dependent relaxation.

Effects of DITPA on the peripheral circulation undoubtedly contributed to the increase in cardiac index. Thyroid hormone is known to decrease systemic vascular resistance and venous compliance while increasing mean circulatory filling pressure and the pressure gradient for venous return (the gradient from peripheral veins to right atrium). Both experimental and theoretical results suggest that the decrease in arterial resistance probably does not play as large a role in increasing cardiac output in hyperthyroidism as once was supposed. For example, raising the peripheral resistance to the euthyroid level with phenylephrine decreases cardiac output by only a small percent. The failing heart is more dependent on afterload than normal, however, and it is conceivable that the tendency of DITPA to reduce peripheral resistance may have contributed to the afterload-reducing effect of captopril in the infarcted animals. Thus, the increase in cardiac index observed with DITPA is likely to have multiple causes, including changes in myocardial contractility, increased venous return, and decreased peripheral vascular resistance. Regardless of the exact contribution of each factor, it is clear that the combination of DITPA and captopril caused a marked increase in cardiac index and decrease in LV EDP.

Structural and Metabolic Effects of DITPA

Evidence of LV remodeling, as measured by ex vivo pressure-volume relations, chamber stiffness, and cavity-to-wall volume ratios, was not greater with DITPA/captopril treatment than with captopril. Earlier studies using a similar period of captopril treatment also did not find changes in LV remodeling in rats with large infarctions (>45%). In rats made hyperthyroid by treatment with T4 for 8 to 10 days, increased LV chamber stiffness and a shift of the pressure-volume relation toward the pressure axis were observed. Similar effects were not seen with the dosage of DITPA used in this study. In addition, hypertrophy (increase in LV mass) was not observed in the group treated with DITPA/captopril. This may be related to the relatively low dose of DITPA used, the less potent effects of DITPA on myocyte growth, or the fact that captopril is known to attenuate LV hypertrophy in rats after large myocardial infarctions.

Because of the possibility that a thyroid hormone analogue might elevate MVO2, an estimate of MVO2 was made on the basis of hemodynamic parameters. Such estimates have been found to correlate surprisingly well with measured values over a wide range of hemodynamic conditions, including infusion of isoproterenol. In the present study, there were no statistical differences in calculated MVO2 among the three groups studied. The mean value for estimated MVO2 was lower for the captopril-treated group compared with estimates of MVO2 in infarcted control rats. This occurred because all three of the parameters used to estimate MVO2, that is, heart rate, LV peak systolic pressure, and \( \frac{+dP}{dt_{\text{max}}} \), were lower with captopril treatment than in the control group. With the addition of DITPA to captopril treatment, the estimated MVO2 increased, but only to the level of untreated control hearts. Thus, captopril mildly decreased estimated MVO2, but did not augment cardiac index compared with controls. The combination of DITPA and captopril, however, markedly improved cardiac performance and lowered LV EDP without an accompanying increase in estimated MVO2 above the value found in the untreated control group.

DITPA Effects on Myocardial Contractility and Relaxation

The direct myocardial effects of DITPA are likely to be a major underlying reason for the improvement seen in cardiac performance of the postinfarction model. This may be explained by the effects of thyroid hormone on intracellular Ca2+ handling, which has been shown to be defective in failing myocardium. In isolated chick myocytes, elevated concentrations of thyroid hormone have been reported to stimulate the rate of flux in and out of the rapidly exchangeable Ca2+ pool, and this is associated with enhanced contractile force. The hyperthyroid heart has been reported to have enhanced sarcoplasmic Ca2+-ATPase, an increased number of Na+1,K+-ATPase sites, and increased Ca2+ uptake by isolated sarcoplasmic reticulum (SR), all of which could be involved in enhanced contractile performance. In rat ventricular myocytes, the amplitude of the Ca2+
transient has been reported to be higher and the rate of decay more rapid in the hyperthyroid state. Furthermore, studies on excised papillary muscles from animals treated with thyroid hormone suggest that there are parallel changes in twitch tension development and the intracellular Ca\(^{2+}\) transient.\(^{17,18}\) Thus, the mechanisms by which myocardial contractility is enhanced by thyroid hormone include alterations in Ca\(^{2+}\) handling by the SR and changes in sarcoplasmic function that could directly influence the excitation-contraction coupling process.

The transcription of several genes involved in Ca\(^{2+}\) handling within the heart has been reported to be decreased in patients with end-stage heart failure of diverse pathogeneses. The genes include those encoding the SR Ca\(^{2+}\)-ATPase,\(^{41}\) SR Ca\(^{2+}\) release channel (ryanodine receptor),\(^{42}\) and the plasma membrane dihydropyridine receptor (Ca\(^{2+}\) channel).\(^{43}\) Defective Ca\(^{2+}\) handling by isolated SR and decreased transcription of the mRNA encoding SR Ca\(^{2+}\)-ATPase also has been found in pressure-induced cardiac hypertrophy in the rat.\(^{44}\) It is noteworthy that expression of some of the same genes, including the SR Ca\(^{2+}\)-ATPase\(^{45}\) and the Ca\(^{2+}\) release channel,\(^{46}\) is increased by T\(_4\) and presumably would be enhanced by T\(_3\) analogues. The possibility that DITPA may reverse some of the biochemical defects associated with the failing heart deserves further investigation.

Several studies have demonstrated the ability of thyroid hormone to stimulate cardiac myosin ATPase activity in the rat and rabbit by shifting the relative proportion of myosin isoforms such that the high-activity V\(_1\) form predominates.\(^{11}\) Although these studies initially led to the hypothesis that enhancement of myocardial contractility by thyroid hormones might be the result of increased myosin ATPase activity, more recent evidence suggests that this is unlikely.\(^{47}\) Specifically, in the rat postinfarction model, it has been shown that hemodynamic improvement occurs within 72 hours of thyroid hormone administration and precedes upregulation of cardiac myosin isoenzymes.\(^{48}\)

Additional evidence indicates that the effects of T\(_4\) on myocardial contractility are not likely to be mediated by the \(\beta\)-adrenergic system. Increases in cardiac \(\beta\)-adrenergic receptor density are reported to occur with T\(_4\) administration.\(^{48,49}\) However, the increase in receptors is not accompanied by a corresponding increase in cardiovascular sensitivity to \(\beta\)-agonists.\(^{50,51}\) Furthermore, the inotropic and hypertrophic effects of the hormone are attenuated minimally, if at all, by \(\beta\)-adrenergic blockade.\(^{52,53}\)

The explanation for the lack of effect of DITPA on heart rate compared with the marked positive chronotropic effect observed with \(\t\)-thyroxine remains unknown. Competition binding studies with \(^{[125]}\)iodothyronine indicate that DITPA binds with similar affinities to the \(\alpha\)- and \(\beta\)-subtypes of the thyroid hormone nuclear receptor.\(^{24}\) Possibly, the more lipophilic character of DITPA may alter its distribution within the heart and other tissues.

**Limitations**

In this study, DITPA was used as adjuvant treatment to captopril, precluding any direct comparisons between DITPA and captopril as single agents. This study design is justified and clinically relevant because ACE inhibition is currently the most effective medical treatment for heart failure after myocardial infarction. The beneficial effects in the DITPA/captopril group are not likely to be related solely to captopril, however, because earlier work has demonstrated minimal direct effects of captopril on contractile performance in papillary muscles of animals given a similar course of treatment.\(^{55}\)

Although the results demonstrate improvements in LV performance with DITPA/captopril treatment, it remains to be established whether this hemodynamic benefit will translate into improved survival with heart failure after myocardial infarction. Earlier studies have shown that cardioactive agents can both improve hemodynamics and prolong survival, however, suggesting that these two end points are not mutually exclusive.\(^{1,56}\) Also, it is well established that activation of the renin-angiotensin system and other neurohormonal pathways is an important consideration in the treatment of heart failure, but the effects of DITPA on these pathways is beyond the scope of the present study.

Since M\(\text{VO}_2\) was not measured directly, it is possible that DITPA may increase energy utilization by processes that are not reflected in mechanical measures of LV performance. Previous studies have demonstrated that M\(\text{VO}_2\) in experimental hyperthyroidism correlates well with the maximum rate of tension development in papillary muscles, however, with increased basal metabolism accounting for no more than 10% to 11% of the increase in energy demand.\(^{57}\) Although it is conceivable that uncoupling of oxidative phosphorylation may have occurred with DITPA treatment, this phenomenon has been described only at extremely high doses of T\(_4\) (4 to 8 mg per day).\(^{58,59}\) which would be equivalent to approximately a 500-fold greater dose of DITPA than was administered.

**Conclusions**

This study demonstrates improvement in LV function with the addition of DITPA, a thyroid hormone analogue, to captopril treatment in rats with infarct-induced heart failure. DITPA improves cardiac performance by a combination of actions, including effects on the heart and peripheral circulation, that are thought to be mediated by binding to high-affinity nuclear receptors and alterations in gene transcription. Currently, none of the agents used for the treatment of heart failure regulate gene transcription or alter protein synthesis within the intracellular milieu of the failing myocardium. Because of this unique mechanism of action, DITPA and perhaps other thyroid hormone analogues offer hypothetical advantages over current inotropic agents by promoting the coordinated synthesis of myocardial proteins involved in intracellular Ca\(^{2+}\) movement and the excitation-contraction coupling process.

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