The novel effects of 3,5,3'-triiodo-L-thyronine on myocyte contractile function and β -adrenergic responsiveness in dilated cardiomyopathy

Medical management of patients with chronic left ventricular dysfunction continues to be a difficult problem. Recent clinical and experimental studies have suggested that 3,5,3'-triiodo-L-thyronine improves left ventricular pump function. However, whether 3,5,3'-triiodo-L-thyronine directly improves myocyte contractile function in cardiomyopathic states is unknown. Accordingly, this study examined the direct effects of 3,5,3'-triiodo-L-thyronine on isolated myocyte contractile function in cardiocytes obtained from control ($n = 6$) pigs and pigs with tachycardia-induced dilated cardiomyopathy (atrial pacing at 240 beats/min for 3 weeks; $n = 6$). Myocyte percent shortening and velocity of shortening were obtained at baseline and in the presence of 3,5,3'-triiodo-L-thyronine doses of 80 and 100 pmol/L. For both control and dilated cardiomyopathy groups, 3,5,3'-triiodo-L-thyronine caused a significant increase in myocyte contractile function. For example, a 100 pmol/L dose of 3,5,3'-triiodo-L-thyronine increased myocyte velocity of shortening by 51 % in control myocytes and by 54 % in dilated cardiomyopathy myocytes compared with baseline. A second series of experiments was performed to determine whether 3,5,3'-triiodo-L-thyronine altered the responsiveness of the β -adrenergic receptor system in control and dilated cardiomyopathy myocytes. Myocyte contractile function was examined during β -adrenergic stimulation with isoproterenol alone and in myocytes preincubated with 3,5,3'-triiodo-L-thyronine doses of 80 and 100 pmol/L to which isoproterenol was added. Isoproterenol alone increased velocity of shortening by 139 % in control and by 233 % in dilated cardiomyopathy myocytes compared with baseline. This was significantly greater than the increase with 3,5,3'-triiodo-L-thyronine alone. 3,5,3'-triiodo-L-thyronine followed by isoproterenol increased velocity of shortening by 245 % in control and 313 % in dilated cardiomyopathy myocytes compared with baseline. This was significantly greater than the response with 3,5,3'-triiodo-L-thyronine or isoproterenol alone and appeared to be greater than an additive response. The results from this study clearly demonstrated that 3,5,3'-triiodo-L-thyronine directly augmented myocyte contractile function in both control and dilated cardiomyopathy myocytes. In addition, 3,5,3'-triiodo-L-thyronine enhanced the contractile response to β -adrenergic stimulation in dilated cardiomyopathy. This study provides unique evidence to suggest that 3,5,3'-triiodo-L-thyronine may be a useful adjunct to conventional inotropic support in the setting of advanced left ventricular dysfunction. (J THORAC CARDIOVASC SURG 1994;108:672-9)

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Supported by National Institutes of Health grant HL45024 (F.G.S.), Established Investigator Award of the American Heart Association (F.G.S.), and Nina S. Braunwald Research Fellowship of the Thoracic Surgery Foundation for Research and Education (J.D.W.).

Received for publication Feb. 28, 1994.

Accepted for publication April 18, 1994.

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Parmacologic management of patients with left ventricular (LV) dysfunction continues to be a difficult problem both in the setting of chronic congestive heart failure and myocardial ischemia, as well as after cardiac operations. Inotropic agents used to improve contractile function include digitalis, β -adrenergic receptor agonists, and phosphodiesterase inhibitors. However, the inotropic

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0022-5223/94 \$3.00 + 0 12/1/57171

effectiveness of these agents appears diminished in patients with chronic LV dysfunction.¹ These limitations may result from the fact that these inotropic agents depend on intact sarcolemmal receptor transduction systems to exert their positive inotropic effects. However, chronic LV dysfunction as seen in cardiomyopathic disease is associated with alterations in sarcolemmal receptor systems and therefore may reduce the effectiveness of these agents.² For example, patients with chronic LV dysfunction exhibit changes in the myocardial sarcolemmal β -adrenergic receptor system.¹⁻⁴ Specifically, Bristow² reported down regulation of β -adrenergic receptors, as well as alterations in adenylate cyclase activation, in patients with dilated cardiomyopathy (DCM). These alterations in the β -adrenergic receptor system in patients with chronic LV dysfunction result in depressed responsiveness to administration of β -adrenergic agonists.¹ Because β -adrenergic agonists are commonly used clinically to augment depressed LV performance, particularly after cardiopulmonary bypass, this diminished responsiveness is clinically important. Recent studies suggest that T_3 , which may exert its effects independent of sarcolemmal receptor systems, may acutely alter β -adrenergic responsiveness, may augment LV contractile function, and may provide a unique mode of inotropic therapy in patients with chronic LV dysfunction. Recent clinical and experimental studies have suggested that acute administration of 3,5,3'-triiodo-L-thyronine (T_3) improves LV pump performance in the setting of LV dysfunction.⁵⁻⁹ For example, Morkin and associates¹⁰ reported that treatment with thyroid hormone for 3 days in a postinfarction model in rats produced a positive inotropic response. In addition, Hammond and coworkers¹¹ reported myocardial β -adrenergic receptor up regulation and increased adrenergic sensitivity in pigs given short-term T_3 treatment. However, it remains unclear whether T_3 acts directly on the myocyte, whether T_3 acts independently of or synergistically on the β -adrenergic receptor system in tachycardia-induced DCM, and the extent to which T_3 augments contractile function in DCM. Therefore, the overall goal of the present study was to examine the direct effects of acute administration of T_3 on isolated myocyte contractile function in a model of DCM.

Methods

Twelve weight-matched pigs (Yorkshire strain, 28 kg) were randomly assigned to one of two groups: (1) pigs subjected to supraventricular pacing tachycardia at 240 beats/min for 3 weeks ($n = 6$) and (2) sham-operated control pigs ($n = 6$). The pacing protocol was performed as previously described.^{12,13} In brief, a stimulating electrode was sutured onto the left atrium and pacemakers were implanted and modified for programming

heart rates up to 400 beats/min (Spectrax, Medtronic, Inc., Minneapolis, Minn.). Seven to 10 days after recovery from the surgical procedure, atrial pacing at 240 beats/min was initiated. Electrocardiograms were obtained frequently during the pacing protocol to ensure the presence of 1:1 conduction. The sham-operated control pigs were cared for in identical fashion with the exception of the pacing protocol. All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.¹⁴

On the day of study, the animals were sedated with 10 mg of midazolam (Versed, Hoffman-La Roche, Inc., Nutley, N.J.), were placed in a custom-designed sling that allowed the animal to rest comfortably, an electrocardiogram was established, and the pacemaker was deactivated (pacing group only). Two-dimensional and M-mode echocardiographic studies (ATL Ultramark VI, 2.25 MHz transducer, Bothell, Wash.) were used to image the LV from a right parasternal approach. Echocardiographic measurements were performed as previously described.^{12,13,15} Next, the animals were anesthetized with isoflurane (0.5%/1.5 L/min), and their lungs were ventilated through a nonrecirculating anesthesia circuit. A sternotomy was then performed, and the heart was quickly extirpated and placed in an oxygenated Krebs solution. The LV and septum were quickly weighed. The region of the LV free wall comprising the left circumflex coronary artery was dissected free, the artery cannulated, and the tissue prepared for myocyte isolation.

Myocyte isolation and contractile function measurement.

By means of methods described by this laboratory previously,^{16,17} oxygenated modified Krebs solution containing aerobic substrates and collagenase (0.5 mg/ml, type II; 146 U/mg, Worthington, Pa.) was perfused and recirculated through the cannulated circumflex artery for 20 minutes. The tissue was then minced into 2 mm sections and added to an oxygenated trituration solution containing calcium chloride 400 μ mol/L and collagenase (0.5 mg/ml). The tissue and trituration solution were transferred to a centrifuge tube and gently agitated. At 15-minute intervals, the supernatant was removed and filtered and the cells were allowed to settle. The myocyte pellet was then resuspended in standard culture media (media 199: Nutrient Mixture F-12, Ca^{2+} 2 mmol/L, Gibco Laboratories, Grand Island, N.Y.).

Isolated myocytes were placed in a thermostatically controlled chamber (37° C) fitted with a coverslip on the bottom for imaging on an inverted microscope (Axiovert IM35, Zeiss Inc., Oberkochen, Germany). The volume of the chamber was 2.5 ml and contained two stimulating platinum electrodes. The myocytes were imaged with a 20 $\times$ long-working-distance Hoffmann Modulation Contrast objective (Modulation Optics Inc., Greenvale, N.Y.). Myocyte contractions were elicited by field stimulating the tissue chamber at 1 Hz (S11, Grass Instruments, Quincy, Mass.) and imaged with a charge-coupled device with noninterlaced scan rate of 240 Hz (GPCD60, Panasonic, Secaucus, N.J.). Myocyte motion signals were captured with the cell parallel to the video raster lines, and this video signal was input through an edge detector system (Crescent Electronics, Sandy, Utah). The distance between the left and right myocyte edges were converted into a voltage signal, digitized, and input to a computer (80286/ZBV2526, Zenith Data Systems, St. Joseph, Mich.) for subsequent analysis. Stimulated myocytes were allowed a 5-minute stabilization period after which contraction data for each myocyte were recorded from a minimum of 20 consecutive contractions. Parameters comput-

Table I. Left ventricular function after chronic supraventricular tachycardia

	Control	DCM
LV weight/body weight (gm/kg)	3.0 ± 0.2	3.1 ± 0.4
Intrinsic heart rate (beats/min)	109 ± 6	153 ± 8*
End-diastolic dimension (cm)	3.4 ± 0.2	5.1 ± 0.3*
Fractional shortening (%)	30 ± 2	10 ± 3*
Sample size (n)	6	6

DCM, Dilated cardiomyopathy after 3 weeks of supraventricular tachycardia (240 beats/min).

**p* < 0.05 versus control.

ed from the digitized contraction profiles included percentage shortening, peak velocity of shortening peak velocity of lengthening total contraction duration, and time to peak contraction.

Experimental design. Myocytes from each control and paced pig were randomly assigned to the following treatment groups: (1) a T₃ dose of 80 pmol/L (Sigma Chemical Company, St. Louis, Mo.), (2) a T₃ dose of 100 pmol/L, and (3) an isoproterenol dose of 25 nmol/L. This concentration of isoproterenol has been reported previously to be the maximum effective dose for this myocyte preparation.^{16,17} Additional groups included (4) a T₃ dose of 80 pmol/L followed by an isoproterenol dose of 25 nmol/L and (5) a T₃ dose of 100 pmol/L followed by an isoproterenol dose of 25 nmol/L. Baseline measurements were obtained for each cell before the addition of either T₃ or isoproterenol. Preliminary studies conducted by this laboratory using increasing concentrations of T₃ (0.01 to 0.5 nmol/L) were performed to generate a dose-response curve. From these dose-response measurements, the effective concentration of T₃ for a 50% response was computed to be 80 pmol/L, and the effective concentration for a 100% response was computed to be 100 pmol/L. These concentrations of T₃ were used throughout the present study. A minimum of 4 cells were assigned to each treatment group for each pig.

Data analysis. LV function was compared between the two groups by means of Student's *t* test (Table I). The steady-state myocyte function was analyzed at baseline and after treatment with T₃, isoproterenol, or both T₃ and isoproterenol by analysis of variance. If the analysis of variance revealed significant differences, pairwise mean differences were compared by means of Bonferroni-adjusted significance levels.¹⁸ To determine whether T₃ had a synergistic effect on β-adrenergic responsiveness with isoproterenol, we examined the interactive effects of T₃ and isoproterenol by means of analysis of variance with a fixed-effects model. All statistical procedures were performed with the use of the BMDP statistical software package (BMDP Statistical Software Inc., Los Angeles, Calif.). Results are presented as mean ± standard error of the mean. Values of *p* < 0.05 were considered to be statistically significant.

Results

LV function with pacing-induced cardiomyopathy. All of the pigs subjected to 3 weeks of pacing-induced supraventricular tachycardia survived the pacing protocol and were found to have symptoms of dyspnea and

Table II. Effects of T₃ on myocyte function

	Baseline	T ₃ , 80 pmol/L 80pMT ₃	T ₃ , 100 pmol/L
Percent shortening (%)			
Control	4.6 ± 0.1	6.2 ± 0.2†	6.4 ± 0.2†
DCM	2.2 ± 0.1*	2.7 ± 0.1*	3.1 ± 0.2*
Shortening velocity (μm/sec)			
Control	45.1 ± 1.5	61.3 ± 2.7†	73.5 ± 3.2†
DCM	29.2 ± 1.3*	43.7 ± 3.3*†	42.3 ± 3.6*†
Lengthening velocity (μm/sec)			
Control	50.8 ± 1.9	72.1 ± 3.3†	80.4 ± 3.2†
DCM	28.9 ± 1.5*	40.8 ± 3.8*	46.1 ± 3.7*†
Time to peak contraction (msec)			
Control	285.1 ± 6.5	309.4 ± 9.8	300.6 ± 11.9
DCM	256.0 ± 7.3*	229.0 ± 12.4*	262.2 ± 14.9*
Duration of contraction (msec)			
Control	530.4 ± 9.0	553.6 ± 18.2	527.6 ± 19.4
DCM	493.4 ± 15.8	466.4 ± 32.0	489.3 ± 27.8

All values are presented as mean ± standard error of the mean. T₃, 3,5,3'-Triiodo-L-thyronine; DCM, dilated cardiomyopathy after 3 weeks of pacing at 240 beats/min.

**p* < 0.05 versus control.

†*p* < 0.05 versus baseline.

tachypnea at terminal study. An LV echocardiographic study was performed on each pig at terminal study with results summarized in Table I. Chronic pacing-induced supraventricular tachycardia caused LV dilatation and dysfunction consistent with results previously reported by this laboratory.^{12,13,19} At autopsy, the pigs subjected to pacing exhibited bilateral pleural effusions and ascites. Thus, consistent with previous reports from this laboratory,^{12,13,19} chronic pacing-induced tachycardia caused clinical and functional manifestations of a DCM. These pigs will be referred to as the DCM group.

Steady-state myocyte contractile function. A high yield (>70%) of viable (rod shaped; quiescent in culture) myocytes was isolated from each control pig and each pig with tachycardia-induced DCM used in this study. Non-stimulated, resting length for control myocytes was 139.1 ± 1.9 μm and for DCM myocytes was 184.4 ± 3.8 μm (*p* < 0.05). This increase in isolated myocyte length is consistent with past reports in this model of DCM.^{12,13} Baseline steady-state measurements of isolated myocyte contractile function in the control group and in the group with DCM are summarized in Table II. A significant depression in baseline indices of myocyte contractile function was observed in DCM myocytes as compared with controls, consistent with previous studies.¹² For example, baseline myocyte percent shortening was 52% lower and velocity of shortening was 35% lower in DCM myocytes than in controls. Thus, as previously reported, DCM myocytes exhibited significant abnormalities in basal steady-state contractile function.^{12,19}

Fig. 1. Effect of T_3 100 pmol/L on the percent and velocity of myocyte shortening for control and DCM myocytes. Data are expressed as percent change from baseline. T_3 caused an equivalent increase in myocyte percent and velocity of shortening in both control and DCM myocytes.

Fig. 2. Effect of T_3 and isoproterenol on myocyte percent and velocity of shortening for both control and DCM myocytes. Data are expressed as percent change from baseline. T_3 and isoproterenol caused an equivalent increase in percent and velocity of myocyte shortening in both control and DCM myocytes. Note that the scale for the y axis differs for the two figures.

Myocyte contractile function with T_3 . With the short-term addition of T_3 in concentrations of either 80 or 100 pmol/L, indices of myocyte contractile function significantly increased from baseline values for both control and DCM myocytes (see Table II). For example, in control myocytes, a 100 pmol concentration of T_3 increased myocyte percent shortening by 38% and increased veloc-

ity of shortening by 51%. In DCM myocytes, similar directional changes in myocyte contractile function occurred. In the presence of T_3 , myocyte contractile function in DCM remained lower than contractile function in control myocytes treated with the same dose of T_3 . However, the percent increase in contractile function caused by T_3 was equivalent in DCM and control myo-

Table III. The effects of isoproterenol and T₃ on myocyte function

	Baseline	Isoproterenol (25 nmol/L)	Isoproterenol (25 nmol/L) + T ₃ (80 pmol/L)	Isoproterenol (25 nmol/L) + T ₃ (100 pmol/L)
Percent shortening (%)				
Control	4.5 ± 0.1	8.8 ± 0.6†	11.4 ± 0.5†‡	12.2 ± 0.5†‡
DCM	2.1 ± 0.1*	4.7 ± 0.5*†	6.2 ± 0.5*†‡	5.8 ± 0.6*†
Shortening velocity (μm)				
Control	45.0 ± 1.3	100.5 ± 7.2†	162.1 ± 8.9†‡	166.3 ± 7.4†‡
DCM	28.8 ± 1.3*	74.3 ± 8.4*†	151.6 ± 13.5†‡	117.1 ± 13.2*†‡
Lengthening velocity (μm)				
Control	50.2 ± 1.7	90.9 ± 6.5†	157.2 ± 11.2†‡	162.4 ± 10.4†‡
DCM	27.6 ± 1.3*	56.1 ± 7.8*†	122.4 ± 18.0*†‡	100.3 ± 14.9*†‡
Time to peak contraction (msec)				
Control	285.6 ± 6.1	232.1 ± 11.2†	218.9 ± 8.3†	241.8 ± 14.2†
DCM	257.0 ± 6.4*	218.9 ± 12.1†	162.1 ± 7.5†	175.3 ± 8.6*†
Duration of contraction (msec)				
Control	532.0 ± 8.5	511.2 ± 23.1	465.8 ± 25.9†	474.3 ± 24.4†
DCM	498.1 ± 13.1	500.9 ± 30.0	393.4 ± 21.5	415.0 ± 20.9†

All values presented as mean ± standard error of the mean. T₃, 3,5,3'-Triiodo-L-thyronine; DCM, dilated cardiomyopathy after 3 weeks of pacing at 240 beats/min.

**p* < 0.05 versus control.
†*p* < 0.05 versus baseline.
‡*p* < 0.05 versus isoproterenol alone.

cytes (Fig. 1). Thus acute administration of T₃ improved indices of myocyte contractile function in both control and DCM myocytes. Furthermore, the percent increase produced by T₃ is comparable between control and DCM myocytes.

Myocyte contractile function with T₃ and β-adrenergic responsiveness. Isoproterenol increased myocyte contractile function in both control and DCM myocytes, as in previous studies (Table III).¹⁶ Isoproterenol increased myocyte velocity of shortening by 139% in control myocytes and by 233% in DCM myocytes over baseline values (Fig. 2). However, the absolute values for percent and velocity of shortening remained significantly below control values. Diminished β-adrenergic responsiveness with development of DCM is consistent with previous reports from this laboratory and others.^{1-4, 20}

Incubation with T₃ followed by the addition of isoproterenol significantly increased indices of myocyte contractile function to a greater extent than isoproterenol alone or T₃ alone for both control and DCM myocytes. For example, in control myocytes preincubation with a 100 pmol/L dose of T₃ followed by the addition of isoproterenol increased velocity of shortening by 246% above baseline values (see Table III). Similarly, DCM myocytes incubated with T₃ 100 pmol/L followed by isoproterenol exhibited a 368% increase in velocity of shortening over baseline values. Thus short-term administration of T₃ followed by β-adrenergic receptor stimulation

caused increased myocyte contractile function in both control and DCM myocytes that was greater than that seen with either agent (T₃ or isoproterenol) alone.

Analysis of variance was performed to determine whether the results obtained with the addition of T₃ followed by isoproterenol were due to additive effects, synergistic effects, or both. This analysis revealed that (1) T₃ significantly affected myocyte contractile function in both control and DCM myocytes and (2) T₃ administration followed by β-adrenergic receptor stimulation significantly enhanced myocyte contractile function over and above isoproterenol alone and T₃ alone. Furthermore, the analysis revealed a significant interaction between administration of T₃ followed by isoproterenol and the treatment groups, that is, control versus DCM (F value = 2.64; *p* = 0.02). That is, the effects of T₃ followed by isoproterenol were significantly different in the DCM group than in the control group. In the control group, T₃ exerted its influence independent of the β-adrenergic receptor system. In DCM myocytes, T₃ was synergistic with the β-adrenergic receptor system such that β-adrenergic responsiveness to isoproterenol significantly increased in more than an additive manner.

Discussion

The purpose of this study was to examine the direct effects of acute T₃ administration on isolated myocyte contractile function in a model of DCM induced by

supraventricular tachycardia. This study demonstrated that the acute administration of T_3 (1) increased isolated myocyte contractile function, (2) increased β -adrenergic responsiveness in both control and DCM myocytes, and (3) selectively enhanced β -adrenergic responsiveness in DCM myocytes. Results from the present study suggest that acute administration of T_3 may provide a unique method of increasing contractile function in the setting of chronic LV dysfunction.

Historically, the effects of thyroid hormone (T_3) on LV function have generally been thought to require modulation of contractile protein synthesis through binding of intranuclear receptors.²¹ These alterations in protein synthesis, however, clearly do not explain the mechanism for the short-term effects of T_3 on myocyte contractile function. In the present study, myocyte contractile performance increased within 30 seconds of T_3 administration. Thus an alternative mechanism for the short-term effects of T_3 other than intranuclear binding must be operative. Dudley and Baumgarten²² recently demonstrated that increased bursting of sodium channels in isolated rabbit myocytes occurred within 30 seconds of acute administration of T_3 to the extracellular face of the sarcolemma. Thus T_3 may acutely increase intracellular sodium and exert a positive inotropic effect via the Na^+/Ca^{2+} exchanger. Rudinger and associates²³ reported an acute increase in Ca^{2+} adenosinetriphosphatase activity in rabbit sarcolemmal preparations in the presence of thyroid hormone. Chang and Kunos²⁴ reported that acute administration of T_3 to rat myocardium caused a significant increase in the β -adrenergic receptor density. The present study demonstrated that T_3 acutely augmented β -adrenergic responsiveness in isolated cardiomyopathic myocytes. Thus a potential mechanism for the enhanced β -adrenergic responsiveness of DCM myocytes to acute administration of T_3 may be an increase in β -adrenergic receptor density. On the basis of the results of the present study, future studies that examine the short-term effects of T_3 on basic myocyte contractile properties and receptor systems may be appropriate.

Clinical reports have documented that patients with chronic LV dysfunction exhibit depressed levels of the active form of thyroid hormone, T_3 .^{5, 6, 25-27} Specifically, Hamilton and colleagues²⁵ reported an association between a depressed level of T_3 and indices of LV function. In a second report, Hamilton²⁶ presented evidence suggesting a relationship between decreased levels of T_3 and survival rates in patients with LV dysfunction. Bucino and coworkers²⁸ reported that the maximum velocity of isotonic shortening in feline papillary muscles varied directly with the thyroid state. Thus past clinical and experimental studies have suggested that T_3 appears

to be an important determinant in overall LV performance.²⁵⁻²⁷ The present study demonstrated that acute administration of T_3 increased indices of myocyte contractile function in the control state and with the development of DCM. Results from the present study provide direct evidence for the contributory role of T_3 in the modulation of contractile function.

Novitzky and associates⁸ reported that acute administration of T_3 led to hemodynamic improvement and reduced need for inotropic support in patients with LV dysfunction after cardiopulmonary bypass. In an experimental study, these same investigators also observed improved cardiac output in dogs subjected to 15 minutes of myocardial ischemia followed by the acute administration of T_3 .^{5, 8, 29} Morkin and colleagues¹⁰ reported increased rate of rise of LV pressure after 3 days of thyroxine administration in rats with chronic LV dysfunction produced by coronary artery ligation. These past clinical and experimental studies suggested that T_3 had beneficial effects when administered acutely in the setting of acute LV dysfunction or for longer periods (3 days) in rats with chronic LV dysfunction. However, no study has directly examined the effects of the acute administration of T_3 on isolated myocyte contractile performance in the setting of cardiomyopathic disease. The present study, for the first time, directly demonstrated that acute administration of T_3 improved indices of isolated myocyte contractile function after the development of DCM.

β -Adrenergic receptor agonists are commonly used as inotropic agents in the setting of LV dysfunction. The effects of these agonists are dependent on intact sarcolemmal receptor transduction systems. However, Bristow² has reported down regulation and uncoupling of the β -adrenergic receptor system in patients with chronic LV dysfunction. This laboratory and others have reported that DCM is associated with similar alterations in the β -adrenergic receptor system.³⁰⁻³² Specifically, pacing-induced DCM in swine or dogs caused a 25% to 57% decrease in total β -adrenergic receptor density.^{1, 30, 32} The past reports in this model of pacing-induced DCM also show decreased β -adrenergic responsiveness.^{1, 30, 32} These changes in the β -adrenergic receptor density and β -adrenergic responsiveness with pacing-induced DCM are similar to those that have been observed in patients with chronic LV dysfunction.^{2-4, 20} Results from the present study demonstrated that β -adrenergic responsiveness was decreased in the DCM myocytes consistent with these past reports.³⁰⁻³² Hammond,¹¹ Williams,³³ Bilezikian³⁴ and others have suggested that a relationship exists between administration of T_3 , or the thyroid state, and the β -adrenergic receptor system. More recently, acute administration of T_3 has been shown to acutely alter the

number of cardiac β -adrenergic receptors.²⁴ The present study therefore examined myocyte contractile function with the concomitant administration of T_3 and a β -adrenergic receptor agonist in this model of DCM. Results from the present study demonstrated that acute administration of T_3 increased β -adrenergic responsiveness in DCM myocytes. Thus these results suggest that acute administration of T_3 may have a beneficial interaction with β -adrenergic agonists in the setting of cardiomyopathic disease.

Previous experimental studies have examined the ability of various inotropic agents to improve contractile function in this model of DCM.^{12, 35} Specifically, this laboratory has demonstrated that the responsiveness of DCM myocytes to increased extracellular Ca^{2+} was significantly blunted.¹² Furthermore, this laboratory reported a reduction in cardiac glycoside receptor density and decreased responsiveness to the positive inotropic effects of the glycoside ouabain.³⁵ Finally, previous studies, as well as the present study, have demonstrated blunted β -adrenergic responsiveness with DCM.^{1, 30, 32} Unlike these past reports, however, the present study clearly demonstrated that the concomitant administration of T_3 and the β -adrenergic receptor agonist isoproterenol exhibited a synergistic effect on myocyte contractile function in DCM myocytes. Thus, for the first time, an inotropic regimen has been demonstrated to improve myocyte contractile function with tachycardia-induced DCM.

Significant differences exist between this *in vitro* model and the effects of T_3 given either acutely or chronically *in vivo*. First, T_3 has multiple systemic effects *in vivo* such as induction of catecholamine release and alteration of heart rate. The present study examined the effects of T_3 on myocyte contractile function *in vitro*, in an environment independent of these extracellular influences. Thus any secondary effects of T_3 on isolated myocyte contractile function could not be determined from the present study design. Second, complete delivery of T_3 to the myocyte was achieved in the present study, which may not be the case *in vivo*. Finally, this study examined the acute effects of T_3 on myocyte contractile performance with a single dose, but did not address the effects of repetitive or chronic administration. On the basis of the results of the present study, which demonstrated that acute administration of T_3 increased isolated myocyte contractile performance in both control and DCM myocytes, further studies are warranted to examine the effects of T_3 *in vivo*, with chronic dosing and with different dosing regimens.

Congestive heart failure remains a major cause of morbidity and mortality in patients with advanced left ventricular dysfunction. Because of the complexity in-

involved in the management of these patients, alternative pharmacologic therapy continues to be investigated. Recent clinical and experimental reports have documented improved indices of LV function after T_3 administration *in vivo*.^{5, 8} The present study, however, demonstrated for the first time that acute administration of T_3 exerted a positive effect on myocyte contractile function in a model of cardiomyopathic disease. In addition, the present study provided evidence that T_3 acutely influenced the β -adrenergic receptor system, thus improving the responsiveness of these cardiomyopathic myocytes to β -adrenergic receptor stimulation. Results from this study suggest that continued investigation of the possibility that acute administration of T_3 may be a useful therapeutic adjunct in the treatment of patients with LV dysfunction is warranted.

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