Cellular and molecular remodeling in a heart failure model treated with the β-blocker carteolol

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FURAZOLIDONE-INDUCED CARDIOMYOPATHY in turkeys is a well-characterized animal model of dilated cardiomyopathy (DCM) with significant similarities to human heart failure (11, 14, 23–25, 27, 31, 36, 38, 40) at both the cellular and organ levels. The observed abnormalities in DCM include ventricular dilatation, thinning of the left ventricular (LV) free wall, and impairment of systolic function. Histologically, there is hypertrophy of cardiac myocytes and enlargement of nuclei as well as reorientation of subepicardial fibers, reflecting increased end-diastolic volume and wall stress (11, 21, 22, 27). Recently, Tominaga et al. (59) also demonstrated that carteolol prevents the development of DCM induced by the encephalomyocarditis virus in a murine model. All of these experimental studies have tested the efficacy of DCM in turkey poults when given concurrently with furazolidone (23). Treatment of furazolidone-induced DCM and spontaneous DCM in turkey poults with propranolol significantly decreased LV diameter and increased free wall thickness (21, 22, 27). The use of β-blockers in the treatment of hypertension, coronary artery disease, and arrhythmias (1, 5, 9, 13, 16, 17, 30, 34, 47, 48, 50, 62, 66). A number of clinical studies have recently shown that β-blockers have long-term beneficial hemodynamic effects in patients with heart failure (1, 5, 9, 13, 16, 17, 30, 34, 47, 48, 50, 62, 66). In a multicenter trial, the β1-selective antagonist metoprolol was found to reduce the combined end point of death and the need for transplantation in patients with idiopathic DCM (61). Another large multicenter clinical trial, in which the nonselective β-blocker carvedilol was used in patients with heart failure, showed an ~68% reduction in mortality (48). However, the underlying mechanisms of the beneficial effects of β-blockers in heart failure remain unsolved.

The use of β-blockade in animal models of heart failure has been investigated, but the results have been ambiguous. For example, in the Syrian hamster model of cardiomyopathy, β-blocker treatment showed no benefit in preventing the development of heart failure (37, 57). Instead, α-receptor blockade was shown to affect the development of cardiomyopathy, suggesting a role for α-receptor-mediated effects in the development of the disease (37, 57). In a different model (hypertensive rats), β-blockers not only controlled blood pressure effectively but also prevented the development of myocardial lesions (15, 33, 58). Our past studies have shown that β-blockade with propranolol is cardioprotective and prevents the development of DCM in turkey poults when given concurrently with furazolidone (23). Treatment of furazolidone-induced DCM and spontaneous DCM in turkey poults with propranolol significantly decreased LV diameter and increased free wall thickness (21, 22, 27). Recently, Tominaga et al. (59) also demonstrated that carteolol prevents the development of DCM induced by the encephalomyocarditis virus in a murine model. All of these experimental studies have tested the efficacy of β-blockade in preventing the development, but not the treatment, of DCM. We were therefore interested in testing the effects of carteolol, a nonselective β-blocker with sympathomimetic effects, in the treatment and potential reversal of established DCM.

The primary objective of this study was to establish the effects of two dosages of carteolol on clinical signs and LV function in animals with advanced heart failure. The second objective was to evaluate the effects of carteolol on survival. The third objective was to examine the cellular and molecular changes induced by carteolol in this model of heart failure.
MATERIALS AND METHODS

Experimental Design

One-day-old broad-breasted white turkey poults were wing-banded for easy identification. At 7 days of age, they were randomly divided into two groups using a random number generator. The control group was maintained on a normal ration, free from any additives, and the experimental group was fed 700 parts per million furazolidone for 2 wk. Consequently, the furazolidone-fed animals developed severe heart failure. At day 21, control animals were randomized into three groups: one group received no pharmacological agent (ConL; n = 25), whereas the other two groups received different concentrations of carteolol, ConL (n = 24) and ConH (n = 27), where the subscripts L and H represent two selected dosages of carteolol, 0.01 (low) and 10 mg/kg body weight (high), respectively. Furazolidone-fed animals were assessed echocardiographically for evidence of cardiac dilatation and reduced ejection fractions (n = 117). Furazolidone-treated animals stopped receiving furazolidone after day 21, and they were similarly randomized into three groups: one group did not receive carteolol treatment (DCM; n = 37), whereas the other two groups, DCML (n = 40) and DCMH (n = 40), were treated with carteolol in the same dosages as respective aged-matched control animals.

Drug Dosage and Administration

The two dosages (0.01 and 10 mg/kg) used in the study were chosen after testing for physiological effects on heart rate and blood pressure. We selected concentrations of carteolol that either did not affect heart rate and blood pressure (low dose) or that only reduced heart rate acutely (high dose). This study enabled us to test the following hypotheses: 1) that carteolol improves myocardial function independent of hemodynamic effects and 2) that reducing the heart rate may prove beneficial for cardiac function.

Serial Tests

An earlier dose-range study was performed in control animals in which more than six different dosages of carteolol were tested. As a result of these studies, we selected the two experimental dosages of carteolol used in this study (0.01 and 10 mg/kg). The low dose of carteolol (0.01 mg/kg) did not affect heart rate or blood pressure at any of the time points measured (data not shown). Administration of the high dose of carteolol (10 mg/kg) resulted in a reduction of heart rate by ~9% with full recovery to baseline within 4 h after dosing. Blood pressure was not affected at this concentration. Serial functional assessment in the study included 1) clinical history and observations, 2) blood pressure determinations, 3) echocardiograms, and 4) body weight gain. Technicians making the hemodynamic measurements were blinded to treatment groups.

Physiological Measurements

DCM animals received an initial dose of 2.5 mg/kg that was well tolerated. This dosage was titrated up to the full concentration over 4 days in increments of 2.5, 5.0, 7.5, and 10 mg/kg. Thigh cuff systolic blood pressure and heart rate were measured noninvasively using a transcutaneous Doppler technique in resting animals (23, 26, 29). Echocardiograms were obtained, as previously reported (26, 29, 31), in nonseated animals with a Portable Interspec Cardioscan.

Gross Morphology and Heart Volumes

The thickness of the LV wall was measured at the level of the mitral valve. The hearts were arrested in diastole, and a volume was recorded. There was a good correlation between heart volumes determined in vivo and in vitro with an R value of 0.96 (data not shown).

Histopathology

The diameters of individual myocytes were measured as previously reported (24). Semi-quantitative point-counting technique was used to quantify connective tissue content in trichrome-stained sections (24). The cell width obtained in isolated control cells (8.7 ± 0.30 µm) correlated well with the measurement of cross-sectional diameter (9.61 ± 0.58 µm) that we obtained (P = 0.2).

Langendorff Heart Preparation

Randomly selected animals from each group at 49 days of age were first heparinized and then anesthetized with pentobarbital sodium. The heart was immediately weighed, attached to a perfusion apparatus, and retrogradely perfused through the aorta (i.e., the Langendorff mode) at constant pressure (~100 mmHg) as previously reported (40). Experiments were performed at 41°C.

Isolated Muscle Preparations

LV trabeculae caeae were dissected from the LV with fiber diameters <750 µm. One end of the muscle was attached to a force transducer in a temperature-regulated bath at 37°C as previously reported (23, 31). Muscles were stimulated to contract using threshold voltage delivered through a punctuated electrode to avoid catecholamine release (3). Muscles from DCM hearts were exposed to increasing concentrations of carteolol (1 × 10⁻⁹ to 1 × 10⁻⁴ M).

Radioligand Binding Studies

Membrane preparation. Samples were weighed and minced with scissors in an ice-cold preparation buffer (10 mM Tris, 1 mM EGTA, pH 8.0). The tissue was homogenized with a Polytron (Brinkman Instruments, Westbury, NY), and contractile proteins were extracted using KCl (500 mM). The fraction was then washed three times in the buffer. After centrifugation at 4,000 g, the pellet was resuspended in a preservation buffer (50 mM Tris, 250 mM sucrose, 1 mM EGTA, pH 7.5). Protein concentration was measured using the Lowry method.

Characterization of β-adrenergic receptors. β-Adrenergic receptor density was measured by [125I]iodocyanopindolol (125IYP) in membrane preparations as described previously (6). A standard curve of five increasing concentrations of 125IYP (specific activity 2,200 Ci/mM) between 25 and 300 pM was used. 125IYP binding was carried out in the presence or absence of 1 µM I-propranolol to construct specific binding curves. The incubation buffer contained 150 mM NaCl, 20 mM Tris, and 1 mM ascorbic acid, pH 7.5. To further characterize β-adrenergic receptors, competition radioligand binding studies in turkey myocardium were performed in Tris-Mg²⁺, pH 7.5. The β-adrenergic receptor antagonists (carteolol, metoprolol, propranolol, and CGP-20712A) were used to displace 125IYP. All measurements were made at steady state at 30°C. Slope of the competition curve, IC₅₀ and the percentage of receptors in high- or low-affinity states were determined by computer modeling (7).

Saturation radioligand binding. Total β-adrenergic receptor content (B_max) and dissociation constants (K_d) were obtained as previously described (7) by performing saturation
Curves with increasing (125ICYP). B\text{max} and K\text{d} were determined by nonlinear least-squares fit of the specific binding curve. B\text{max} was expressed relative to the total protein content of the sample as femtomoles per milligram of protein. \(\beta_1\) and \(\beta_2\)-receptor percentages were determined by the displacement of bound 125ICYP (50 pM) by CGP-20712A (1 \mu M) or propranolol (1 \mu M). \(\beta_1\) and \(\beta_2\)-receptor densities (fmol/mg protein) were calculated by applying \(\beta\)-receptor percentages to the total \(\beta\)-receptor density (B\text{max}).

Competitive radioligand binding. Competition binding data was obtained from the displacement of 50 pM 125ICYP by \(\beta\)-adrenergic receptor antagonist agents (carteolol, metoprolol, propranolol, and CGP-20712A). Slope and IC\text{50} values were obtained.

The percentages of fitted \(\beta_1\) and \(\beta_2\)-receptors were determined by computer modeling of the ICYP-CGP-20712A competition curve.

Adenylyl Cyclase Activity

Adenylyl cyclase activity was assayed as described previously (8). Briefly, 75–250 \mu g of membrane protein were diluted in a buffer solution containing (in mM) 100 Tris, 0.1 Mg-ATP, 0.5 MgCl\text{2}, 0.01 GTP, 1 cAMP, 10 phosphocreatine, and 14.5 \mu g creatine kinase (CK), with pH 7.3 at 30°C. After a 5-min preincubation period, measurements of adenylyl cyclase activity were initiated by the addition of 1–2.5 \mu Ci of [\alpha-32P]ATP (NEN, Boston, MA).

Metabolic Enzyme Activities

Before the assay was performed, 100 mg of myocardium were first diluted in 9 volumes of 80 mM KCl, 50 mM Tris, and 40 mM NaN\text{3} at pH 7.2. This preparation was homogenized for three intervals of 10 s separated by 30-s rest periods using a tissue homogenizer and was then centrifuged for 10 min in a centrifuge at 1,500 \times g. The supernatant was removed, and CK, lactate dehydrogenase (LDH), aspartate transaminase (AST), and myoglobin were determined as previously described (2, 44, 45, 51).

Determination of Sarcoplasmic Reticulum Ca\text{2+} Cycling

Sarcoplasmic reticulum (SR) Ca\text{2+} uptake and Ca\text{2+}-release channel (CRC) activities of myocardial homogenates were determined in real-time using fluorescence spectrofluorometry and the fluorescent Ca\text{2+}-indicator dye indo 1 as described by O’Brien and co-workers (12, 41, 43, 45). A 5-min incubation of the homogenate in the presence of 500 \mu M ryanodine has been previously shown to lock the CRC in a closed confirmation (19, 45), whereas brief exposure locks the CRC open (46). In addition to the measured indicators of Ca\text{2+}-cycling activity, unidirectional CRC activity was derived by computerized subtraction of the rate of free Ca\text{2+} concentration (18) versus time with the CRC open from the corresponding record obtained after a 5-min preincubation in 500 \mu M ryanodine (Ca\text{2+}-pump activity).

Preparation of Myofibrils and Myofibrillar Mg-ATPase Activity

The LV of hearts from each group were used to prepare myofibrils according to Solaro et al. (56). Myofibrillar Mg-ATPase activity was determined from measurements of inorganic phosphate (P\text{1}) according to the method described by King (39).

Myocardial ATPase Activities

ATPase activities of myocardial homogenates were determined using methods previously described (43, 45, 55, 56). The ATPase reaction was initiated by the addition of Na\text{2}ATP and MgCl\text{2}. The Ca\text{2+}-ATPase activity of the SR, determined in the same homogenates as other enzymatic assays, is the azide-insensitive ATPase activity that is inhibited by 20 mM Ca\text{2+} (45). The Ca\text{2+}-ATPase of the SR is specifically inhibited when extravesicular Ca\text{2+} increases to millimolar levels due to saturation of the low-affinity Ca\text{2+} binding site on the enzyme. Specificity for the SR Ca\text{2+}-ATPase was achieved by exploiting this back-inhibition phenomenon. Measurement of the SR Ca\text{2+}-ATPase activity as the activity specifically inhibited by millimolar Ca\text{2+} eliminates potential interference from any other Ca\text{2+}-stimulated ATPase in the assay system (45). Mitochondrial F1-ATPase activity, determined in the same homogenates as the total ATPase, is the ATPase activity that is inhibited by 40 mM NaN\text{3} (45). In vivo, the F1-ATPase operates as the mitochondrial ATP-synthase.

Chemicals

Carteolol hydrochloride was obtained from Otsuka Pharmaceutical (Tokushima, Japan). All other chemicals were of reagent grade or better and were purchased from Sigma Chemical (St. Louis, MO).

Statistical Analysis

Data were represented as means ± SE for continuous variables. The proportion of animals surviving at 49 days in the treatment group was compared by Fisher’s exact test. The distributions of the continuous variables were checked for normality. Student’s t-test was used to compare means of normally distributed continuous variables. Parametric one-way analysis of variance (ANOVA) techniques were used to compare normally distributed continuous variables among the different groups. Differences among the six groups were assessed using ANOVA. The Fisher’s protected least-significant difference test, Dunnett’s t-test, and Schéffe’s multiple-comparison test were used accordingly. A value of P < 0.05 was considered significant. Unpaired, two-tailed t-tests were done on certain groups to evaluate statistical differences, and a value of P < 0.05 was considered significant.

Table 1. In vivo baseline assessment at 21 days of age

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HR, beats/min</th>
<th>SBP, mmHg</th>
<th>RPP, mmHg/min</th>
<th>EF, %</th>
<th>HW, g</th>
<th>BW, g</th>
<th>HW/BW</th>
<th>ESV, ml</th>
<th>EDV, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>13</td>
<td>367 ± 8.0</td>
<td>155 ± 6.0</td>
<td>56,885 ± 48.0</td>
<td>88.5</td>
<td>4.6</td>
<td>719</td>
<td>6.4 × 10^-3</td>
<td>0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>DCM</td>
<td>9</td>
<td>348 ± 7.0</td>
<td>71* ± 4.0</td>
<td>24,708* ± 28.0</td>
<td>28.3*</td>
<td>5.02</td>
<td>395*</td>
<td>12.7 × 10^-3*</td>
<td>1.15*</td>
<td>1.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = no. of control (Con) or furazolidone-fed animals with dilated cardiomyopathy (DCM). Animals were subsequently euthanized for in vitro baseline assessment. HR, heart rate; SBP, peak systolic blood pressure; RPP, rate-pressure product; EF, ejection fraction; HW, heart weight; BW, body weight; ESV, end-systolic volume; EDV, end-diastolic volume. *P < 0.01, significantly different from Con.
In vivo characteristics at 49 days of age

intrinsic heart rate or blood pressure. After 1 wk of treatment, the effects of carteolol on the intrinsic heart rate and blood pressure were minimal in both DCM and control animals. The low dose (0.01 mg/kg) of carteolol exerted no significant effect on the intrinsic heart rate or blood pressure. After 1 wk of treatment, an acute decrease in heart rate (~9%) was observed 30 min after the high dose (10 mg/kg) of carteolol was administered. Heart rate remained depressed for ≤4 h after dosing before returning to the initial depressed baseline.

Baseline Assessment

The data summarized in Table 1 show that the mean body weight of DCM animals was significantly decreased and that the ratio of heart to body weight was significantly increased compared with that in the control animals. Ejection fraction was 28.3 ± 4.0% in the DCM group. DCM animals had a fourfold enlargement in LV heart volume. Although there were no differences in heart rates between the control and DCM groups, in Langendorff-perfused hearts peak LV systolic pressure (LVSP) of DCM hearts was 24 ± 4 mmHg, which was only 22% of that obtained from control hearts (108 ± 6.5 mmHg). The reduced LVSP (~79%) resulted in a large difference in rate-pressure product (RPP) between the two groups of hearts.

Follow-Up and Outcome Measurements in Subgroups

Mortality. During treatment, all animals tolerated each dosage of carteolol. Follow-up ended after 28 days of treatment or no treatment with carteolol (age 49 days). After 4 wk of treatment, 22 of 37 animals died in the nontreated DCM group (59% mortality). Twenty-two of forty animals died in the group treated with a low dose of carteolol (55% mortality), and only nine of forty died in the group treated with a high dose of carteolol (22% mortality). Demonstrated in Fig. 1 are survival curves for all three treatment groups. There was no survival benefit in the low-dose group (P = 0.8184). However, there was a significant improvement in survival in the high-dose group (P = 0.00132). Control animals not receiving treatment and control animals receiving only carteolol demonstrated 100% survival (n = 25 Con; n = 24 ConL; n = 27 ConH).

Ejection fraction. As an assessment of in vivo cardiac function, we obtained echocardiograms after treatment. As shown in Table 2 and Fig. 2, ejection fraction 28 days after randomization was decreased by 63 and 41% in nontreated DCM and DCML animals compared with baseline. There was progressive dilatation of LV volume in nontreated DCM animals 28 days after randomization (at age 49 days). In DCML animals, LV size increased only to 55% of LV size in nontreated DCM animals, whereas in DCMH animals, LV length and weight were similar to those of control animals (Table 3).

Table 2. In vivo characteristics at 49 days of age

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HR, beats/min</th>
<th>SBP, mmHg</th>
<th>RPP, mmHg/min</th>
<th>EF, %</th>
<th>BW, g</th>
<th>HW, g</th>
<th>HW/BW, ×10⁻³</th>
<th>ESV, ml</th>
<th>EDV, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>25</td>
<td>±8</td>
<td>±5</td>
<td>±40</td>
<td>±0.6</td>
<td>±81</td>
<td>±0.81</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.02</td>
</tr>
<tr>
<td>ConL</td>
<td>24</td>
<td>±7</td>
<td>±6</td>
<td>±42</td>
<td>±2.5</td>
<td>±132</td>
<td>±2.64</td>
<td>±0.2</td>
<td>±0.05</td>
<td>±1.96</td>
</tr>
<tr>
<td>ConH</td>
<td>27</td>
<td>±11</td>
<td>±3</td>
<td>±33</td>
<td>±1.5</td>
<td>±147</td>
<td>±1.47</td>
<td>±0.1</td>
<td>±0.10</td>
<td>±0.16</td>
</tr>
<tr>
<td>DCM</td>
<td>15</td>
<td>±24</td>
<td>±6</td>
<td>±144</td>
<td>±2.5</td>
<td>±92</td>
<td>±2.76</td>
<td>±0.3</td>
<td>±0.65</td>
<td>±0.33</td>
</tr>
<tr>
<td>DCML</td>
<td>18</td>
<td>±279</td>
<td>88†</td>
<td>24.552†</td>
<td>16.6†</td>
<td>±104</td>
<td>±104</td>
<td>±0.3</td>
<td>±0.44</td>
<td>±0.5</td>
</tr>
<tr>
<td>DCMH</td>
<td>31</td>
<td>±12</td>
<td>±8</td>
<td>±96</td>
<td>±4.1</td>
<td>±104</td>
<td>±104</td>
<td>±0.3</td>
<td>±0.44</td>
<td>±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = no. of animals. Con, nontreated control animals; ConL and ConH, control animals treated with low (0.01 mg/kg) and high (10.0 mg/kg) doses of carteolol; DCM, nontreated animals; DCMH, DCM animals treated with low and high doses of carteolol. * P < 0.05, significantly different from Con group. † P < 0.05, significantly different from DCM group.
significant improvement in body weight (an increase of 24% compared with the nontreated DCM group). The high dose of carteolol resulted in a 45% increase in body weight compared with the nontreated DCM group.

At 49 days of age there was no difference in the ratio of heart weight to body weight for the three control groups (Table 2). The ratio of heart weight to body weight for the DCM group was highest for nontreated DCM animals, intermediate for DCM_L, and lowest for the DCM_H group (Table 2). The high dose resulted in a significant reduction in heart-to-body weight ratios compared with nontreated DCM animals, but these ratios were not different from those for the control group.

At the end of the study, gross and histopathological studies were performed on the LV, right ventricle (RV), and interventricular septum (IVS). LV diameter and length and LV, RV, and IVS weights were significantly greater in the nontreated DCM animals, and there was a significant thinning of the LV free wall (Table 3). The high dose of carteolol significantly reduced LV diameter and length, increased LV free wall thickness, and decreased RV and IVS weight compared with values in the nontreated DCM group (Table 3). In DCM_L hearts, compared with nontreated DCM hearts, there was significant improvement in LV diameter as well as a decrease in LV length. RV and IVS weight were not different from those in nontreated DCM animals.

At the cellular level, DCM hearts exhibited hypertrophy with enlarged myocyte fiber diameters (Table 3). DCM animals that received the high dose of carteolol, however, had normal myocyte fiber diameters. In cross section, DCM hearts demonstrated a diffuse interstitial fibrosis. With the use of a semiquantitative point-counting technique, connective tissue content was found to be significantly increased in DCM hearts compared with that in the control groups. Carteolol decreased the amount of connective tissue in the treated DCM hearts at both dosages.

β-Adrenergic Receptors in Turkey Myocardium

β-Adrenergic receptors in control turkey myocardium were characterized by competition binding experi-

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Table 3. Gross morphological parameters at 49 days of age

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LV_D, mm</th>
<th>LV_L, mm</th>
<th>LV_W, mm</th>
<th>LW, g</th>
<th>RV, g</th>
<th>IVSW, g</th>
<th>Fiber Diameter, µm</th>
<th>Connective Tissue Content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>25</td>
<td>13.0</td>
<td>27.0</td>
<td>8.7</td>
<td>7.9</td>
<td>2.0</td>
<td>3.7</td>
<td>9.61</td>
<td>3.0</td>
</tr>
<tr>
<td>Con_L</td>
<td>24</td>
<td>±1.4</td>
<td>±0.8</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.1</td>
<td>±0.2</td>
<td>±0.58</td>
<td>3.1</td>
</tr>
<tr>
<td>Con_H</td>
<td>27</td>
<td>±1.0</td>
<td>±1.0</td>
<td>±0.4</td>
<td>±1.3</td>
<td>±0.3</td>
<td>±0.9</td>
<td>±0.78</td>
<td>±0.80</td>
</tr>
<tr>
<td>DCM</td>
<td>15</td>
<td>±1.2</td>
<td>±0.9</td>
<td>±0.3</td>
<td>±0.6</td>
<td>±0.2</td>
<td>±0.3</td>
<td>±0.46</td>
<td>±0.7</td>
</tr>
<tr>
<td>DCM_L</td>
<td>18</td>
<td>±1.0</td>
<td>±0.9</td>
<td>±0.3</td>
<td>±0.8</td>
<td>±0.2</td>
<td>±0.3</td>
<td>±0.62</td>
<td>±0.9</td>
</tr>
<tr>
<td>DCM_H</td>
<td>31</td>
<td>±1.4</td>
<td>±1.6</td>
<td>±0.2</td>
<td>±0.9</td>
<td>±0.4</td>
<td>±0.2</td>
<td>±0.58</td>
<td>±1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = no. of hearts. LV_D, left ventricular length; LV_L, left ventricular diameter; LV_W, left ventricular free-wall thickness; LVW, left ventricular weight; RV, right ventricular weight; IVSW, interventricular septum weight. For heart weights, n = 5 because other hearts were freeze-clamped for biochemistry (30). *P < 0.05, significantly different from Con group. †P < 0.05, significantly different from DCM group.
Influence of Long-Term β-Blockade

Total β-adrenergic receptor density (percentage of B_max) in hearts from nontreated DCM animals (45 fmol/mg protein) was reduced by 50% compared with Con animals (88 fmol/mg protein) (Fig. 3). DCM_L and DCM_H treatment increased the β-adrenergic receptor density by 29 and 20 fmol/mg protein, respectively. Treatment with carteolol did not affect total β-adrenergic receptor density in control myocardium (Fig. 3).

In hearts from nontreated DCM animals, density of β₁-adrenergic receptors decreased by ~75% compared with that in control myocardium from nontreated animals (Con). The β₁-adrenergic receptor density increased significantly compared with the nontreated DCM group in myocardium from the carteolol-treated DCM groups (54% in DCM_L, 69% in DCM_H).

The β₁-adrenergic receptor percentage dropped by 47% in myocardium from nontreated DCM animals, increased by 24% in the DCM_L group, and increased by 52% in the DCM_H group. The β₂-receptor density was not changed in hearts from DCM animals.

Adenylyl Cyclase Activity

Because we had observed that treatment with the high dose of carteolol improved cardiac function to a greater extent than with the low dose, we measured adenylyl cyclase activity in hearts from animals receiving long-term high-dose carteolol treatment. We investigated whether the restoration of function by long-term high-dose carteolol treatment is accompanied by an increase in adenylyl cyclase activity and coupling to β-adrenergic receptors. In hearts from nontreated DCM animals, the basal adenylyl cyclase activity was decreased by 30% compared with that in the nontreated Con group. Long-term treatment with the high dose of carteolol was also associated with decreased basal adenylyl cyclase activity in Con_H (60%) and DCM_H hearts (40%) compared with that in the nontreated groups.

Long-term treatment with carteolol in control and DCM hearts did not affect stimulation of adenylyl cyclase by isoproterenol, NaF, or forskolin. Although 5′-guanylyl imidodiphosphate [Gpp(NH)p] stimulation was decreased in DCM_H versus DCM hearts, this effect was also observed in Con_H compared with Con hearts and is apparently due to the pharmacological effects of carteolol (Table 4).

Metabolic Markers

We used biochemical markers to estimate the capacities of key metabolic pathways as follows: 1) oxidative phosphorylation using mitochondrial ATP synthase and myoglobin, 2) ATP regeneration using CK, 3) mitochondrial NADH shuttle using AST, 4) glycolysis using LDH, and 5) ATP utilization as nonmitochondrial ATP hydrolysis (total ATPase activity). As demonstrated in Table 5, the activities of total ATPase, CK, LDH, AST, ATP synthase, and myoglobin were significantly lower in hearts from nontreated DCM animals compared with these activities in hearts from control

Table 4. Effect of long-term carteolol treatment on adenylyl cyclase activity

<table>
<thead>
<tr>
<th>Adenylyl Cyclase Activity, pmol cAMP · min⁻¹ · mg⁻¹</th>
<th>Basal</th>
<th>Gpp(NH)p</th>
<th>NaF</th>
<th>Iso</th>
<th>Forskolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>6.7 ± 1.4</td>
<td>17.7 ± 2.5</td>
<td>19.7 ± 4.3</td>
<td>10.3 ± 0.9</td>
<td>78.1 ± 8.7</td>
</tr>
<tr>
<td>Con_H</td>
<td>2.7 ± 0.4*†</td>
<td>8.0 ± 1.5*</td>
<td>17.2 ± 1*</td>
<td>6.4 ± 1*</td>
<td>52 ± 3*</td>
</tr>
<tr>
<td>DCM</td>
<td>4.7 ± 0.7*†</td>
<td>8.5 ± 1*</td>
<td>24.1 ± 2.7*</td>
<td>6.4 ± 0.7*</td>
<td>60.9 ± 5.5*</td>
</tr>
<tr>
<td>DCM_H</td>
<td>2.8 ± 0.5*†</td>
<td>4.6 ± 0.3*†</td>
<td>18.5 ± 1.7*</td>
<td>4.9 ± 0.6*†</td>
<td>36.9 ± 2.9*†</td>
</tr>
</tbody>
</table>

Values are means ± SE and were obtained at time of euthanasia after treatment or no treatment with carteolol (age 49 days). Gpp(NH)p, 5′-guanylyl imidodiphosphate; I so, isoproterenol. *P < 0.05, significantly different from Con group. †P < 0.05, significantly different from DCM group.
animals. Treatment with the low dose of carteolol resulted in a significant increase in ATP synthase, myoglobin, and total ATPase activities. In contrast, hearts from animals that received the high dose of carteolol had all markers restored to values observed in control hearts (e.g., total ATPase, CK, LDH, AST, ATP synthase, and myoglobin).

**SR Ca\(^{2+}\)-ATPase Activity and Pumping Rates**

SR Ca\(^{2+}\)-ATPase activity was significantly lower in hearts from nontreated DCM animals compared with that in control animals (3.4 ± 0.6 vs. 11.4 ± 0.7 IU/g, respectively) (Table 5). The SR Ca\(^{2+}\)-pumping rate was significantly lower (24.4 ± 6.3 nM/s) in DCM hearts compared with that in control hearts (41.8 ± 2.1 nM/s). In DCM\(_{H}\) hearts, SR Ca\(^{2+}\)-ATPase activity and SR Ca\(^{2+}\)-ATPase pumping rate were significantly higher than those in nontreated DCM hearts. Although SR Ca\(^{2+}\)-ATPase activity was increased in hearts from animals receiving the high dose, the activity did not reach the levels seen in control myocardium. Treatment of animals with the low dose of carteolol significantly increased SR Ca\(^{2+}\)-ATPase pumping rates; however, SR Ca\(^{2+}\)-ATPase activity was not significantly altered.

**SR CRC Activity**

CRC activity was significantly slower in nontreated DCM hearts compared with that in control hearts (8.2 ± 1.4 vs. 15.9 ± 1.4 nM/s) (Table 5). SR CRC activity in hearts from DCM animals treated with either the low or high dose of carteolol was significantly increased compared with that in nontreated DCM hearts and was restored to levels observed in control hearts.

**SR Net Ca\(^{2+}\)-Sequestration Activity**

Net Ca\(^{2+}\)-sequestration activity was significantly decreased in hearts from nontreated DCM animals compared with that in control hearts. Although SR Ca\(^{2+}\)-ATPase activity was increased in hearts from animals receiving the high dose, the activity did not reach the levels seen in control myocardium. Treatment of animals with the low dose of carteolol significantly increased SR Ca\(^{2+}\)-ATPase pumping rates; however, SR Ca\(^{2+}\)-ATPase activity was not significantly altered.

---

**Table 5. Metabolic enzyme activities and myocardial Ca\(^{2+}\) cycling**

<table>
<thead>
<tr>
<th>Metabolic markers</th>
<th>Control</th>
<th>DCM</th>
<th>DCM(_{L})</th>
<th>DCM(_{H})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK, IU/g</td>
<td>4,500 ± 14 (4)</td>
<td>1,400 ± 129* (9)</td>
<td>2,24 ± 20* (8)</td>
<td>2,74 ± 23* (8)</td>
</tr>
<tr>
<td>LDH, IU/g</td>
<td>275 ± 8 (18)</td>
<td>219 ± 12* (9)</td>
<td>231 ± 17* (9)</td>
<td>275 ± 22* (8)</td>
</tr>
<tr>
<td>AST, IU/g</td>
<td>274 ± 8.2 (17)</td>
<td>187 ± 8.2* (9)</td>
<td>187 ± 8.2* (9)</td>
<td>187 ± 8.2* (9)</td>
</tr>
<tr>
<td>ATP synthase, IU/g</td>
<td>145 ± 4.2 (8)</td>
<td>87 ± 4* (4)</td>
<td>111 ± 7.9* (5)</td>
<td>127 ± 9.3* (5)</td>
</tr>
<tr>
<td>Myoglobin, µg/g</td>
<td>27.2 ± 3.1* (5)</td>
<td>51.7 ± 2.7* (4)</td>
<td>67.2 ± 5.1* (5)</td>
<td>67.2 ± 5.1* (5)</td>
</tr>
<tr>
<td>Total protein, mg/g</td>
<td>128 ± 2.3 (36)</td>
<td>111 ± 3.0* (8)</td>
<td>127 ± 4.7* (8)</td>
<td>125 ± 5.4 (7)</td>
</tr>
<tr>
<td>SR Ca(^{2+}) cycling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(^{2+})-ATPase, IU/g</td>
<td>11.4 ± 0.7 (8)</td>
<td>3.4 ± 0.6* (4)</td>
<td>4.5 ± 0.9* (5)</td>
<td>7.3 ± 0.7* (5)</td>
</tr>
<tr>
<td>Ca(^{2+})-ATPase pump, nM/s</td>
<td>41.8 ± 2.1 (23)</td>
<td>24.4 ± 6.3* (9)</td>
<td>40.9 ± 2.7 (5)</td>
<td>49.9 ± 3.6 (8)</td>
</tr>
<tr>
<td>Ca(^{2+})-release channel, nM/s</td>
<td>15.9 ± 1.4 (22)</td>
<td>8.2 ± 1.4* (9)</td>
<td>13.8 ± 1.4* (5)</td>
<td>17.5 ± 2.1* (8)</td>
</tr>
<tr>
<td>Net Ca(^{2+}) sequestration, nM/s</td>
<td>32.3 ± 1.8 (22)</td>
<td>19.4 ± 1.9* (8)</td>
<td>30.9 ± 4.3 (5)</td>
<td>36.7 ± 2.5* (12)</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses indicate no. of hearts. CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate transaminase. Ca\(^{2+}\)-ATPase activity was normalized per gram of protein. *P < 0.05 compared with Con. †P < 0.05 compared with DCM.
pared with that in hearts from control animals. Treatment with either the low or high dose of carteolol restored net Ca$^{2+}$-sequestration to levels seen in control hearts.

Myofibrillar Mg-ATPase Activity and Myofibril Protein Content

Figure 4 demonstrates the myofibrillar ATPase activity as a function of pCa in hearts from control animals, nontreated DCM animals, and from DCM animals receiving either the low or high dose of carteolol. There was a significant reduction in the maximal myofibrillar ATPase activity in hearts from nontreated animals with DCM (31%) as well as a significant reduction in myofibril protein content (14%) (Table 5). Maximal myofibrillar Mg-ATPase activity was significantly lower in nontreated DCM (72.5 ± 3.8 nmol P$_i$·min$^{-1}$·mg protein$^{-1}$) and DCM$_L$ animals (77.8 ± 2.3 nmol P$_i$·min$^{-1}$·mg protein$^{-1}$) compared with that in hearts from nontreated control animals (110.9 ± 2.9 nmol P$_i$·min$^{-1}$·mg protein$^{-1}$). With low-dose carteolol treatment there was no improvement in Ca$^{2+}$-activated myofibrillar ATPase activity. Hearts from DCM$_H$ animals demonstrated a significant increase in maximal myofibrillar ATPase activity (95.3 ± 2.5 nmol P$_i$·min$^{-1}$·mg protein$^{-1}$) compared with hearts from nontreated DCM and DCM$_L$ animals.

Isolated Muscle Studies

We tested the effects of carteolol in isolated fibers from the LV of nontreated DCM hearts. As shown in Fig. 5, carteolol exerted no significant effects on systolic force. However, there was a decrease in diastolic force when carteolol was added to the bath (Fig. 5B). Isolated muscle preparations from DCM hearts demonstrated a negative force-interval relationship at higher contraction rates, whereas control hearts demonstrated a positive force-interval relationship at 37°C (Fig. 6A). Muscles from the DCM$_H$ group, however, demonstrated a positive force-interval relationship similar to that observed in control hearts (Fig. 6B).

Cardiac Function

To address whether cellular remodeling of metabolic and Ca$^{2+}$-cycling markers seen with carteolol treatment normalizes the frequency response in Langendorff-perfused hearts, peak pressure development was measured from isolated hearts from DCM$_H$, DCM$_L$, and control animals paced at different stimulation rates (Fig. 7). Hearts from nontreated DCM and DCM$_L$ both demonstrated a blunted or negative frequency response, although global myocardial function was improved in the DCM$_L$ group. However, there was a positive frequency response similar to that seen in control hearts in the DCM$_H$ group.

DISCUSSION

Carteolol has beneficial effects that are both heart rate dependent and independent and that occur in the absence of afterload reduction. The DCM group not receiving treatment demonstrated progressive cardiac...
dilatation with a 63% decrease in ejection fraction compared with baseline as opposed to the low-dose carteolol treatment group, which demonstrated only a 41% decrease in ejection fraction compared with baseline. These data support the idea that, at even very low dosages, carteolol slows deterioration of cardiac function. Both the high and low doses of carteolol significantly improved in vivo peak developed pressure, RPP, and LV diameter and free wall thickness, and both induced the regression of myocyte hypertrophy.

**β-Blocker Effects in DCM Animals**

We have previously reported the effects of propranolol, a nonselective β-blocker, atenolol, a β₁-selective blocker, and metoprolol, a β₁-selective blocker (21, 22). We found that gross morphology was improved with all agents but to different degrees. The level of efficacy was propranolol > atenolol ≥ metoprolol. Nonselective β-blockers appeared to have a larger beneficial effect in our avian models, similar to some reports in human heart failure. In the Syrian hamster model and pacing-induced dog heart failure models, β-blockers have been demonstrated to be detrimental (25, 37, 57). However, beneficial effects of β₁-selective and nonselective blockers in the setting of heart failure have been reported in myocarditis-induced DCM in mice (59) and in dogs with LV dysfunction produced by multiple sequential intracoronary microembolizations (53). In dogs with reduced LV ejection fraction, long-term therapy with metoprolol has been reported to prevent the progression of LV systolic dysfunction and LV chamber dilatation. Currently, we have ongoing studies with carvedilol, a nonselective β-blocker with a rank order of potency of β-subtype 1 > α-subtype 1 > β-subtype 2 and with antioxidant properties (67).

**Effects on Heart Rate and Blood Pressure**

At the concentrations studied, we noted no adverse effects on body growth and development. All animals tolerated either dose of carteolol, demonstrating its safety in animals without cardiac dysfunction and in animals with DCM. Blood pressure of the animals receiving either the low or high dose of carteolol remained unchanged throughout this study.

Tachycardia in these animals often results in rapid cardiac decompensation and death. This was observed in severely sick animals subjected to routine blood pressure and echocardiographic measurements. Placing the animal in a recumbent position often resulted in tachypnea with increased respiratory distress, cyanosis, and cardiac decompensation.

**Effect on Myocyte Hypertrophy**

In nontreated DCM hearts and hearts receiving the low dose of carteolol, there was significant myocyte hypertrophy. A very clear distinction seen between the low and high doses of carteolol in treated DCM groups was the regression of hypertrophy in the high-dose group as well as significant remodeling of both the LV and RV. These data would then suggest that regression of hypertrophy and biventricular remodeling is beneficial and associated with improved cardiac performance; the high dosage of carteolol resulted in complete regression of myocyte hypertrophy. One could then speculate that a more favorable mitochondria-to-myofibril ratio was attained in the DCM group. The greater benefits obtained at the high dosage of carteolol are similar to findings in humans showing that patients who received higher dosages of metoprolol and carvedilol benefited most from the therapy (1, 48, 62).

**Effect on Frequency Response**

In isolated muscles from hearts of animals with DCM, increased frequencies of contraction did not improve contractile performance. This phenomenon has been well described in vivo and in vitro (1, 9, 23, 32, 54). At higher frequencies of stimulation, active force decreases in myocardium from failing hearts. This is accompanied by an increase in diastolic force and diastolic Ca²⁺ concentration ([Ca²⁺]). Intracellular [Ca²⁺] is closely related to energy supply in the form of CK activity, ATP concentration, and creatine phosphate concentration (40). An increase in stimulation frequency in failing myocardium results in an increase in energy demand and accumulation of by-products such as ADP, Mg²⁺, Pᵢ, and H⁺, which can have detrimental effects on force generation and on the activity of key enzymes of contraction coupling such as SR Ca²⁺-ATPase (28, 40).

**Effect of Carteolol on Interstitial Fibrosis**

We found a threefold increase in the amount of connective tissue in nontreated DCM hearts compared with control hearts. It has been suggested that perivascular fibrosis of intramyocardial coronary arteries that extend into neighboring interstitial spaces may be responsible for the progression of heart failure (63–65). Treatment with carteolol decreased the amount of connective tissue present. Mechanisms by which a β-blocker can decrease fibrosis could either be a decrease in circulating angiotensin II as a result of improved cardiac performance or a decrease in the expression of myocardial angiotensin-converting enzyme (63–65).

**Effect on β-Adrenergic Receptor Adenylyl Cyclase System**

Turkey β₁-receptors have 82% homology with human β₁-receptors. In the LV myocardium of the nonfailing turkey heart, we measured a β₁-to-β₂-adjrenergic receptor distribution of 75:25%. These findings are similar to human nonfailing myocardium, in which a β₁-to-β₂ ratio of 80:20% has been reported (6, 10, 52). In mammalian myocardium, β-adrenergic receptors are coupled via G proteins to adenylyl cyclase (6, 10, 52). In turkey myocardium, isoproterenol stimulates adenylyl cyclase activity in a manner and extent similar to that seen in human myocardium (4, 8). This provides evidence that avian myocardium also has receptor-mediated stimulation of adenylyl cyclase activity. Fur-
thermore, the response to Gpp(NH)₃ and NaF stimulation suggests evidence for G protein coupling and interaction in avian myocardium similar to that previously described in human myocardium (8).

One of the key abnormalities in human failing myocardium is a selective downregulation of β₂-adrenergic receptors. We observed a selective downregulation of myocardial β₂-adrenergic receptors (~50%). The density of β₂-adrenergic receptors remained unchanged as reported in human myocardium (6, 10). The downregulation of β₂-adrenergic receptors was accompanied by decreased receptor-stimulated adenylyl cyclase activity in failing turkey myocardium, similar to findings in human myocardium (4, 6).

We have shown that β-blocker treatment resulted in a selective remodeling of β₂-adrenergic receptors and did not affect β₁-adrenergic receptors. After long-term treatment with a low or high dose of carteolol, the β₁-adrenergic receptor density in failing myocardium was similar to that in nonfailing myocardium. The increased density of β₂-adrenergic receptors (i.e., β₂-receptors), however, was not accompanied by an increase in adenylyl cyclase activity. Basal adenylyl cyclase activity remained depressed. Long-term treatment with carteolol in control animals also resulted in a decrease in basal adenylyl cyclase activity. However, in control animals that received long-term treatment with carteolol, cardiac function was not affected despite a decrease in adenylyl cyclase activity to levels similar to those in nontreated DCM animals.

In DCM animals, cardiac performance was improved with carteolol treatment even though basal adenylyl cyclase activity remained depressed. These data in both control (Conₙ) and DCM animals may suggest that the adenylyl cyclase system may not be the primary determinant of force-development and contractility. These findings provide evidence that an increased number of β₂-adrenergic receptors in the setting of heart failure does not per se predict automatically an increased responsiveness to catecholamines as demonstrated in the group receiving low-dose carteolol treatment or restored adenylyl cyclase activity as shown in the group receiving high-dose carteolol treatment. This can be due theoretically to an uncoupling of β₂-adrenergic receptors from the stimulatory G protein (4, 8, 20). An alternative explanation for the decrease in adenylyl cyclase activity seen with the high dose of carteolol in the control animals might be that 1) even with extensive washing, there may be some contamination of the membrane preparations by endogenous norepinephrine and 2) inverse agonist effects of antagonists lower basal activity, including activity stimulated by forskolin. The data reported herein demonstrates that cardiac performance cannot be predicted by simply measuring β₂-adrenergic receptor density or adenylyl cyclase activity.

Effects on Enzymatic Activities

Key adaptive changes of the myocardium of failing hearts involve a decrease in enzyme activity of major proteins involved in energy metabolism, e.g., CK, LDH, myofibrillar ATPase, and SR Ca²⁺-ATPase. Markers of energy metabolism on the supply side were favorably affected by carteolol treatment. CK activity (ATP generation), LDH (glycolysis), AST (mitochondrial NADH shuttle), ATP synthase, myoglobin (oxidative phosphorylation), and total ATPase (a marker of nonmitochondrial ATP hydrolysis) were increased in DCMH hearts. Myoglobin (a marker of myocardial respiration), ATP synthase, and total ATPase activities were favorably impacted by treatment with either dose of carteolol. These data indicate that, over a wide dose range, carteolol treatment was (from an energy standpoint) beneficial in animals with overt heart failure. The high dose of carteolol resulted in restoration of all measured markers of energy metabolism (e.g., markers of mitochondrial oxidative phosphorylation and cytosolic glycolysis). LV size was significantly improved with both the high and low doses. We have recently shown by feeding a creatine analog, β-guanidinopropionic acid, that depleting creatine and decreasing CK activity did not result in dilatation and overt heart failure (40).

Instead, cardiac contractile reserve to inotropic and chronotropic stimulation was decreased. Similarly, a significant decrease in contractile reserve to chronotropic stimulation was shown in hearts from animals receiving the low dose of carteolol despite having significantly higher LVSP, ATP synthase, and total ATPase activities. The frequency response was blunted or negative despite significant reduction in LV heart volume and a significantly increased RPP compared with the response in hearts from nontreated DCM animals. The improvement in RPP in the DCMH group reflects an increase in LVSP. These data support the hypothesis that CK plays a key role in contractile response to increased workloads, e.g., inotropic stimulation or tachycardia (28, 40, 42). Although there was structural, functional, and cellular remodeling of the heart with the low dose of carteolol, the response to increases in workload (e.g., heart rate) was not restored. The observation that a higher concentration of carteolol resulted in a greater improvement in cardiac function, positive frequency response, and survival might suggest an additive effect as well as additional cellular remodeling. These data demonstrate a dose-dependent improvement in myocardial energy metabolism and cardiac function.

Effects on Ca²⁺-Regulatory Enzymes

A Ca²⁺-overload state has been linked in theory to reduced energy reserves (28, 43). The accumulation of metabolic by-products at higher workloads as seen with tachycardia or with increased inotropic stimulation can inhibit pumps and ATPases, further driving down in vivo enzyme activities and function of energy supply proteins as well as energy-utilizing proteins (e.g., SR Ca²⁺-ATPase and myofibrillar ATPase). In addition, Mg²⁺, Pi, and H⁺ affect myofilament Ca²⁺ activation. A potential relationship between Ca²⁺ overload and the time course of protein deterioration might also be suggested. With elevated resting [Ca²⁺], proteases are activated and can result in myofilament degradation.
(63). Our data suggest that adaptations in myocardial energy metabolism and Ca\(^{2+}\)-cycling proteins are more quickly restored as opposed to the negative impact at the level of the contractile proteins, i.e., myofibrillar ATPase activity.

On the Ca\(^{2+}\)-cycling side, the SR Ca\(^{2+}\) pumping rate and SR CRC activity were restored to levels seen in nonfailing hearts from DCM\(_L\) or DCM\(_H\) animals. Although the Ca\(^{2+}\) pumping rate was restored with either dose of carteolol, there was no significant improvement in SR Ca\(^{2+}\)-ATPase activity in hearts from animals that received the low dose of carteolol. These data again suggest that normalization of Ca\(^{2+}\)-cycling abnormalities do not per se restore myocardial function or the frequency response (i.e., in the DCM\(_L\) group). Despite restored myocardial contractility in the DCM\(_H\) group, SR Ca\(^{2+}\)-ATPase activity remained significantly less than that seen in nonfailing hearts. These data indicate a dissociation between SR Ca\(^{2+}\) pumping rate and SR Ca\(^{2+}\)-ATPase enzymatic activity in hearts from animals treated with the low or high dose of carteolol.

Correlations have been reported by several investigators among SR Ca\(^{2+}\)-ATPase activity, intracellular [Ca\(^{2+}\)], systolic and diastolic force, and the negative or blunted frequency response. Our findings suggest that in vitro SR Ca\(^{2+}\)-ATPase activity may not reflect Ca\(^{2+}\) pumping rate or net Ca\(^{2+}\) mobilization (23, 32). The determination of the net effect of adaptations at the level of the SR most likely requires in vivo or in vitro dynamic quantitative measurements of intracellular [Ca\(^{2+}\)]. However, it is important to point out that our experiments were performed in the presence of oxalate, which may result in an artificially higher value for Ca\(^{2+}\) mobilization compared with that in the in vivo state. Our data supports the idea of Ca\(^{2+}\)-cycling abnormalities as being important in heart failure. SR Ca\(^{2+}\)-ATPase activity was not significantly increased in hearts from animals that received the low dose of carteolol, yet SR CRC activity and SR Ca\(^{2+}\)-sequestration ability (net Ca\(^{2+}\) sequestered) were restored, most likely as a result of the increased SR Ca\(^{2+}\) pumping rate. The fact that SR Ca\(^{2+}\)-ATPase activity was not increased suggests a relationship between the blunted or negative frequency response and SR Ca\(^{2+}\)-ATPase activity level. Hearts from animals that received the high dose of carteolol demonstrated SR CRC function and net SR Ca\(^{2+}\) sequestration rates not different from those in control hearts, as well as a significant increase in SR Ca\(^{2+}\)-ATPase activity. A balance between SR Ca\(^{2+}\) sequestration and SR CRC activity appears to be important for functional improvement and restoration of the inotropic response to tachycardia.

Hearts with depleted energy reserves demonstrate reduced ability to increase contractility in response to tachycardia (28, 40). Calculations in such hearts of the value of free energy demonstrate that the value approaches the free energy of ATP hydrolysis required for the SR Ca\(^{2+}\)-ATPase pump. This calculation suggests that Ca\(^{2+}\) homeostasis in failing hearts can be affected by decreased energy reserves (28, 40). There was restored CK activity in hearts from animals treated with the high dose of carteolol, which acts as an energy-reserve system. Similarly in these hearts, there was a significant improvement in SR Ca\(^{2+}\)-ATPase activity. This combined restoration of energy reserves and Ca\(^{2+}\) mobilization may have resulted in the reestablishment of a positive frequency response to chronic stimulation.

It is of interest that only the high dose of carteolol significantly increased myofibrillar ATPase activity. Our data suggest that, despite normalization of energy metabolism markers and Ca\(^{2+}\)-cycling parameters, myocardial contractile reserve and response to challenge (tachycardia) involves, at a minimum, partial restoration of myofilament Ca\(^{2+}\) responsiveness and contractile element interactions. Others (49) have suggested that the decrease in cardiac function observed in heart failure is related to the decrease in myofibril protein content. Interestingly, myofibril protein content was not different for the DCM\(_L\) or DCM\(_H\) groups, yet there were significant differences in functional improvement between the two groups. In these DCM groups, there were significant differences in peak LV pressure, RPP, and heart-to-body weight ratios. Although the progression of LV dilatation was slowed by the low dose of carteolol, the hearts continued to dilate, but to a lesser extent than hearts from nontreated DCM animals. As previously reported, the reversal of myocyte hypertrophy was only observed at the high dose of carteolol, yet both doses decreased connective tissue content. Again, changes at the level of the contractile elements would appear to be important because myofibrillar ATPase-pCa response curves from hearts from nontreated DCM or DCM\(_H\) animals, both of which demonstrated blunted or negative frequency responses, were superimposable.

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