Differences in Cardioprotective Efficacy of Adrenergic Receptor Antagonists and Ca$^{2+}$ Channel Antagonists in an Animal Model of Dilated Cardiomyopathy

Effects on Gross Morphology, Global Cardiac Function, and Twitch Force


Turkey poults fed furazolidone (Fz) in high concentrations (700 ppm) develop dilated cardiomyopathy (Fz-DCM). We tested whether five cardiovascular agents were cardioprotective in this model of heart failure, ie, whether they prevented dilatation and wall thinning and improved contractile performance. We compared the effects of chronic administration of a $\beta_1$-selective and a nonselective $\beta$-receptor antagonist, an $\alpha_1$-receptor antagonist, and two Ca$^{2+}$ channel antagonists in the presence of Fz administration. The greatest cardioprotection was found with treatment either propranolol or nifedipine. At the gross morphological level, the effect of propranolol (a nonselective $\beta$-adrenergic antagonist) was greater than the effect of atenolol (a selective $\beta_1$-adrenergic antagonist), and the effect of nifedipine was greater than that of verapamil (Ca$^{2+}$ channel antagonists), with all agents more cardioprotective than phenoxymethylzine (an $\alpha_2$-adrenergic antagonist). Differences in cardioprotective efficacy of each agent increased with increased concentration. These data indicate that the dose and choice of a specific type of Ca$^{2+}$ channel antagonist or $\beta$-receptor antagonist might be important in the treatment of dilated cardiomyopathy. All agents that were cardioprotective caused similar functional improvements at both the whole heart and isolated muscle levels. Compared with control animals, Fz-DCM animals showed a significant reduction in peak left ventricular (LV) developed pressure (92±17 versus 143±24 mm Hg, $P<.05$), +dP/dt (1151±219 versus 2454±549 mm Hg/s), and −dP/dt (1128±291 versus 1875±396 mm Hg/s), with a significant increase in LV end-diastolic volumes (2.8±0.7 versus 0.16±0.1 mL for control animals, $P<.05$). In contradistinction, LV +dP/dt and −dP/dt values for animals receiving Fz plus a cardioactive agent that demonstrated cardioprotection were not significantly different from control values. Peak LV developed pressures were also similar for Fz animals receiving an agent that demonstrated cardioprotection and control animals not receiving any pharmacologic agent. Isolated muscles from Fz-DCM animals as well as animals receiving Fz plus cardioprotective pharmacologic agents responded normally with regard to increasing extracellular Ca$^{2+}$ concentrations. Peak twitch forces were greater for animals receiving cardioprotective agents plus Fz than control animals not receiving any pharmacologic agents or Fz alone. At higher stimulation rates, Fz-DCM muscles demonstrated a significantly reduced peak twitch force (4±0.5 versus 1.5±0.4 g/mm² for control muscles versus Fz-DCM muscles, respectively). The negative effect of higher stimulation rates on peak twitch force was reversed by agents demonstrating the greatest cardioprotection, eg, propranolol and nifedipine. Finally, muscles from hearts treated with agents shown to be cardioprotective in terms of mechanical performance also had a higher tissue content of certain enzymes important for maintaining normal energy (ATP) supply and normal sarcoplasmic reticulum function. These studies indicate that gross morphological changes correlate with contractile performance at the whole heart and isolated muscle level. Because of the different protection provided by drugs from a similar functional class, it is likely that these cardiovascular agents act via mechanisms other than a reduction in heart rate or blood pressure. Rather, we suggest that these agents result in macromolecular remodeling in the myocyte that is conducive to preserved contractile performance. (Circ Res. 1992;73:1077-1089.)

Key Words • turkeys • furazolidone • $\beta$-receptor antagonists • Ca$^{2+}$ channel antagonists • $\alpha_1$-receptor antagonists • cardiomyopathy
An animal model of heart failure that mirrors the human disease state would allow us to distinguish between whether observed abnormalities in end-stage heart tissues are due to heart failure or to the therapy for heart failure. We have recently described the cardiac physiology of the turkey poult and found it to be similar to that of humans and of other nonhuman mammalian species. We have also described the histopathologic similarities between furazolidone (Fz)–induced dilated cardiomyopathy (Fz-DCM) and idiopathic cardiomyopathy seen in humans. Both types of cardiomyopathy histologically demonstrate enlarged bizarre nuclei and hypertrophy of myocytes with reorientation of fibers consistent with increased end-diastolic volume and reduced ejection fractions with resultant hypotension. The turkey poult model provides a controlled setting to evaluate the disease process at various durations and severities with currently used as well as new pharmacologic interventions. In the present study, we asked whether, in this model of cardiomyopathy, there are cardioactive agents that can prevent changes in cardiac function preceding overt dilatation and failure. We have previously reported that propranolol was cardioprotective in turkey poult with Fz-DCM, whereas digitalis, an agent expected to increase [Ca\textsuperscript{2+}], was not cardioprotective, as assessed by morphological measurements of left ventricular chamber diameters, dimension, and wall thickness.4 It has been suggested that some of the beneficial effects derived from β-adrenergic antagonist therapy in human cardiomyopathy result from a reduction in heart rate. However, previous studies have shown that animals given propranolol concomitantly with Fz exhibit no decrease in heart rate.5 Similarly, preliminary experiments in our laboratory have demonstrated resolution of ventricular dilatation in animals with Fz-DCM that received propranolol, independent of any reduction in heart rate or hypotensive effects.6 These results indicate that the efficacy of β-adrenergic antagonist therapy in Fz-DCM is secondary to direct effects on cardiac muscle. Therefore, we investigated whether the protection against the development of cardiomyopathy afforded by propranolol is secondary to the prevention of excess myocardial Ca\textsuperscript{2+} influx. In this regard, we studied different classes of Ca\textsuperscript{2+} channel antagonists (eg, dihydropyridine and phenylalkylamine). We also compared the effect of treatment with the β-selective antagonist atenolol, which should not produce effects on the peripheral vasculature, and propranolol, a nonselective β-adrenergic receptor antagonist. In addition, an α-adrenergic antagonist was studied because of its reported effectiveness in the prevention of the development of dilated cardiomyopathy in the Syrian hamster. Functional effects were assessed at the level of the isolated Langendorff-perfused heart and in isolated muscle preparations. Drug concentrations were used that have been previously demonstrated by us not to affect heart rate or blood pressure.1

Materials and Methods

Gross Morphology Study

In series 1, 120 broad-breasted white turkey poulets (7 days old) were randomized into six groups of 20 birds each. Animals were wing-banded for easy identification. They were housed in heated brooders and were provided with a commercial starter mash and water ad libitum. One group served as controls; the other five groups received 700 ppm Fz in their feed. Of the five groups receiving Fz, one group received no other treatment, while each of the treatment groups received (per 100 g body weight) either propranolol (1 mg three times a day), atenolol (1 mg once a day), nifedipine (1 mg three times a day), or phenoxybenzamine (1 mg once a day). All drugs were given orally with a repeat-dosing micropette. Drug dosages were increased twice a week to compensate for weight gains. After 2 weeks, the birds were lightly sedated with acetylpromazine (0.2 mg/100 g) and ketamine (15 mg/100 g), which previous studies have shown not to affect heart rate or blood pressure.7-9 Heart rates were determined by electrocardiograms. The birds were then weighed and euthanatized with chloroform. The hearts were excised quickly, weighed, and perfusion-fixed with 5% glutaraldehyde. Medial sagittal sections were cut, and thicknesses of the interventricular septum, left ventricular free wall, and lesser diameter of the left ventricular lumen were measured just apical to the mitral orifice and just basilar to the apex of the posterior papillary muscle. The means of each measurement, heart rates, and heart weight to body weight ratios (×100) were calculated for each group and compared by ANOVA. If a suitable F statistic was obtained, individual means were compared by Dunnett’s t test. A value of P < .05 was required before differences between means were considered significant.

The results of series 1 suggested that all treatments exerted some cardioprotective effect, which was manifested by a reduction in the heart weight to body weight ratio and in the left ventricular cavity diameter and by an increase in wall thickness when compared with Fz-DCM animals. However, differences in the degree of cardioprotection were observed among the treatment groups. We hypothesized that our results were skewed by dissimilar therapeutic doses required for each agent rather than actual efficacy. Experiments in series 1 were repeated at higher drug doses after performing toxicity studies (series 2). For this series of experiments, the maximum tolerated dose of β-adrenergic receptor antagonists (3 mg/100 g once a day for atenolol and 3 mg/100 g three times a day for propranolol) was defined as the dose at which sedation, prolonged bradycardia, or hypotension
did not occur. The phenoxybenzamine dose was not increased above 2 mg/100 g once a day because of the profound hypotension associated with larger doses. Verapamil was included as a pharmacologic agent in series 2 because this Ca\(^{2+}\) channel antagonist has been shown to be cardioprotective in the Syrian hamster model of cardiomyopathy.\(^{10}\) Maximal tolerated doses of 4 mg/100 g three times a day of verapamil and 5 mg/100 g three times a day of nifedipine were chosen. Because some of the observed differences might be due to antioxidant properties of some of the agents, we studied a group of animals orally dosed with vitamin C (400 mg once a day), a potent antioxidant.

In series 2, hearts were fixed with 10% buffered formalin before sectioning. Statistical analysis for this series included ANOVA with an algorithm for unequal sample size and the Newman-Keuls multiple sample comparison, with a value of \(P<.05\) considered significant.

**Functional Studies**

In series 3, 1-day-old turkey poults were obtained, fed, and housed as previously described. In this series, we did not choose to study phenoxybenzamine, because it had the least effect in the morphology study. Only agents demonstrated to be cardioprotective in series 1 and 2 were studied. Animals were dosed as described in series 2. The animals serving as controls did not receive any pharmacologic agent, one group received only the pharmacologic agent, one group received a pharmacologic agent plus Fz, and another group received only Fz (Fz-DCM). Dosing of all pharmacologic agents (including Fz) was stopped 8 hours before experimental studies. Hearts were weighed at the time of euthanasia. Hearts from animals randomly selected using a random number generator were studied by Langendorff perfusion or by isolated muscle preparations. Animals were studied after the first and second weeks of treatment to determine if contractile changes occurred before overt dilatation.

**Langendorff Preparation**

Animals were heparinized 5 minutes before being anesthetized. Birds were anesthetized by chloroform and decapitated, and the hearts were excised quickly and placed immediately in an oxygenated ice-cold physiological salt solution. The composition of the bathing medium and perfusion solution was as follows (mmol/L): NaCl, 118; NaHCO\(_3\), 25; KCl, 4.7; CaCl\(_2\), 5.5; MgCl\(_2\), 1.2; EDTA, 0.5; and dextrose, 11. The bathing medium and perfusion solution were oxygenated with 95% O\(_2\)-5% CO\(_2\), pH 7.4. Hearts were then mounted on a constant-pressure Langendorff apparatus (83 mm Hg) and later switched to constant flow. Flow was increased until there was no further increase in peak developed pressure, which was always \(\approx83\) mm Hg. Flow rates were recorded throughout the experiment. A Frank-Starling curve was generated by increasing the volume of a balloon placed in the left ventricle. By using ascending and descending values on the Frank-Starling curve, we were able to obtain two to three measurements at each volume, thereby confirming the stability of our preparations. An apical drain was placed in the left ventricle before insertion of the balloon. Left ventricular end-diastolic volume and pressure, peak developed pressure, and the first derivative of pressure were recorded. Experiments were performed at 41°C and measured in the right ventricle.

All experimental procedures were performed in accordance with the "Position of the American Heart Association on Research Animal Use" (1984) and in accordance with Harvard University’s rules for humane treatment of animals.

**Isolated Muscle Studies**

Birds were anesthetized as described above. Hearts were quickly excised and placed in a bathing medium (see above for composition of medium). Left ventricular trabeculae carneae were dissected free (<250-\(\mu\)m diameter) and placed in temperature-regulated organ baths. Muscles were attached at the base by a small muscle clamp with the other end connected to a force transducer. Experiments were performed at 30°C (unless otherwise noted). Muscles were stimulated to contract using threshold voltage at 1 Hz via a 5-millisecond square-wave pulse (unless otherwise noted) delivered through a punctate electrode located at the base of the muscle. This has been shown to avoid catecholamine release.\(^{11}\) Muscles were allowed to equilibrate for 1 hour, during which time they were stretched to a point at which there was no further increase in active tension. Time to peak tension was measured from the time of initiation of contraction to the peak twitch force. Time to 50% relaxation was measured from the peak tension response. Peak twitch force was normalized for cross-sectional area. Muscles were exposed to increasing Ca\(^{2+}\) concentrations in the bathing medium (2 to 16 mmol/L). The absence of phosphate prevented Ca\(^{2+}\) precipitation. The pH value (7.4) did not vary throughout the experiment.

Means of the parameters from isolated muscle and Langendorff preparations were analyzed by ANOVA, and when indicated, specific means were compared by Newman-Keuls post hoc analysis, requiring a value of \(P<.05\) for significance.

**Muscle Biochemistry Studies**

**Sarcoplasmic reticulum Ca\(^{2+}\) cycling.** Sarcoplasmic reticulum (SR) Ca\(^{2+}\) cycling activity of left ventricular homogenates was estimated by directly monitoring Ca\(^{2+}\) sequestration and Ca\(^{2+}\) release using methods described in detail elsewhere.\(^{12-14}\) Briefly, Ca\(^{2+}\)-sequestration activity of myocardial homogenates from seven control, six Fz-DCM, five propranolol plus Fz–treated, and five nifedipine plus Fz–treated turkey poults was determined using indo 1 ratiometric spectrofluorometry. In this method, the rate of sequestration of extravesicular ionized calcium is monitored in real time. Rates of lowering SR Ca\(^{2+}\) (nanomoles per second) were determined at \(\approx1\) mmol/L Ca\(^{2+}\) at 37°C. The effects of opening and closing the SR Ca\(^{2+}\)-release channel on the rate of Ca\(^{2+}\) sequestration were determined using 500 \(\mu\)mol/L ryanodine, which interacts specifically with the Ca\(^{2+}\)-release channel. Ryanodine was added 10 seconds or 30 minutes before initiating the 160-second reaction to open or close, respectively, the channel. A single isofrom of the ryanodine receptor has been reported in avian myocardium, similar to that found in studies in nonhuman mammals and in humans.\(^{15}\) It appears in preliminary studies that the ryanodine receptor is gen-
were measured in heart tissue in vivo and in vitro) and is the net result of bidirectional Ca²⁺ flux into the SR via the Ca²⁺-ATPase and release through the Ca²⁺-release channel. (2) Ca²⁺-pump activity is the maximal unidirectional sequestration activity of the SR measured when the Ca²⁺-release channel is locked in a fully closed conformation after a 30-minute preincubation with 500 μmol/L ryanodine. (3) Unidirectional Ca²⁺-release channel activity was determined during active Ca²⁺ sequestration as the algebraic difference between Ca²⁺-uptake rates with the Ca²⁺-release channel in a fully closed conformation and when the channel was fully opened by acute exposure to 500 μmol/L ryanodine. (4) Total Ca²⁺ cycling by the SR indicates the amplitude of the Ca²⁺ cycle and was estimated as the algebraic sum of the maximal Ca²⁺-sequestration rate and the rate of Ca²⁺ release.

SR Ca²⁺-ATPase activity was measured using a phosphate-liberation-end point assay to determine ATP-hydrolytic activity that was activated by 1 μmol/L CaCl₂ and inhibited by 20 mmol/L CaCl₂.

The means of each measurement were calculated and compared by ANOVA. When the F statistic indicated, individual means were compared by a Newman-Keuls post hoc analysis with a value of P<.05 considered significant.

Markers of energy supply. Because creatine kinase (CK) and mitochondrial F₁-ATPase (F₁-ATPase) activities have been previously demonstrated to be decreased in Fz-DCM and to contribute significantly to the energy reserve in myocardium, we measured tissue content of these enzymes. CK and F₁-ATPase activities were measured as reported previously in heart samples from control, Fz-DCM, nifedipine plus Fz-treated, and propranolol plus Fz-treated animals. Total creatine content (the sum of phosphocreatine and creatine) was measured as previously described. CK and F₁-ATPase are expressed as international units (IU) or nanomoles per milligram of cardiac protein. The Lowry method was used for protein determination in all experiments.

Statistical Analysis

ANOVA followed by post hoc analysis was used where appropriate. Mean±SD values are presented for all reported data. A value of P<.05 was considered significant.

Chemicals and Reagents

All chemicals and reagents were of the highest analytic grade and were purchased from Sigma Chemical Co, St Louis, Mo.

Results

Morphological measurements obtained from animals in series 1 and 2 are listed in Table 1. Animals euthanatized after 1 week of treatment demonstrated no change in cardiac dimensions (eg, wall thinning or dilatation) for any of the pharmacologic interventions plus Fz or for Fz alone. After 2 weeks of treatment, Fz-treated animals tended to weigh less than control animals. In series 1, control birds weighed significantly more than birds in other groups, and birds fed propranolol plus Fz weighed significantly less. Heart weights of Fz-DCM birds tended to be greater than hearts of control birds or birds receiving therapeutic agents. Heart weights of birds receiving propranolol were less than heart weights of any other group. For series 2, there were no significant differences in body weight for Fz-treated animals except for the propranolol- and nifedipine-treated groups, which demonstrated the greatest cardioprotection. Heart weights of Fz-DCM ani-
mals weighed the most. In both series 1 and series 2, heart weight to body weight ratios were largest for Fz-DCM birds or for phenoxybenzamine plus Fz-treated birds. The mean heart weight to body weight ratio was significantly smaller in the propranolol treatment group and in the median range for birds in the atenolol or nifedipine treatment groups (Table 1, Fig 1D).

With respect to other morphological measurements, birds in all treatment groups in series 1 exhibited some degree of cardioprotection, as evidenced by a reduction in left ventricular cavity diameter (Table 1). In addition, with the exception of the propranolol- or phenoxybenzamine-treated groups, birds from all treatment groups exhibited increases in septal and/or free-wall thickness (Table 1).

Heart rates for birds under light sedation were lowest for Fz-DCM birds (362±55 beats per minute) but did not differ among the other groups of birds (control, 405±36 beats per minute; propranolol, 402±31 beats per minute; atenolol, 379±49 beats per minute; nifedipine, 384±38 beats per minute; and phenoxybenzamine, 388±26 beats per minute). The observed decrease in heart rate in Fz-DCM birds was consistent with previous observations of Simpson et al.21 in this model.

The results of series 2, in which the dose of each pharmacologic agent had been increased, are shown in Table 1. There was no significant difference in the heart weight to body weight ratios of control or Fz-treated birds in series 2 compared with series 1.

In hearts from series 2 birds, the septum and free wall heart measurements of the propranolol, nifedipine, and verapamil treatment groups were thicker than in hearts from the other groups and compared with hearts from Fz-DCM animals (Table 1). Left ventricular cavity diameter was significantly reduced in the propranolol- and nifedipine-treated groups when compared with the Fz-DCM group but not in the atenolol- or phenoxybenzamine-treated groups, despite an increase in the dose of these agents (Table 1). Of note, left ventricular cavity diameters in the atenolol, phenoxybenzamine, and verapamil treatment groups were significantly larger than diameters in the control group.

In series 2, treatment with propranolol appeared to result in greater cardioprotection than treatment with atenolol for all measured parameters and reached statistical significance when comparing left ventricular cavity diameters, heart weight to body weight ratio, and septum and free-wall thicknesses. Similarly, increased cardioprotection was observed with nifedipine treatment compared with verapamil with respect to all measured parameters except the heart weight to body weight ratio.

A comparison of morphological measurements of birds in series 1 and series 2 demonstrates that increasing the dose of propranolol and nifedipine provided greater cardioprotection. For both treatment groups, this comparison reached statistical significance with respect to a reduction in left ventricular cavity diameter (Fig 1A). In addition, there was a significant increase in septal thickness for propranolol-treated and nifedipine-treated birds (Fig 1B) and in free-wall thickness for both treatment groups in series 2 (Fig 1C) when compared with their respective treatment groups in series 1. Nevertheless, no significant difference in the heart weight to body weight ratio for treatment groups in series 2 versus series 1 was observed (Fig 1D). Fig 2 demonstrates the improvement in cardioprotection seen with propranolol and nifedipine by use of an index of dilation of the left ventricle described previously by Einzig et al.17 and calculated as the left ventricular diameter to left ventricular free-wall thickness ratio.
Representative hearts from control and Fz-DCM birds and from birds in each treatment group are shown in Fig 3. This figure illustrates the relative lack of cardioprotection with phenoxybenzamine. This figure also illustrates the difference between hearts from propranolol versus atenolol treatment groups and hearts from the nifedipine versus verapamil treatment groups. A group of birds (n=10) also received 0.05 mg digoxin once a day plus Fz. These animals did not demonstrate cardioprotection with regard to wall thinning, heart weight, or chamber dimension (data not shown). This finding confirms our earlier experimental findings despite an increase in the digoxin dosage.\(^2,3,5\)

Because of the antioxidant properties of propranolol and nifedipine, we studied a group of animals treated with vitamin C. Vitamin C, a potent antioxidant, when given orally (400 mg once a day) was found to be somewhat cardioprotective; heart volumes were as follows: vitamin C alone, 0.44±0.1 mL (n=11); Fz, 2.8±0.7 mL (n=5); and vitamin C plus Fz, 1.4±1.0 mL (n=6), *P*<.03 compared with Fz alone).

Cardiac Function Studies

No change in cardiac function (eg, both whole heart and isolated muscle level) was noted at 1 week after any of the pharmacologic interventions with or without Fz or for Fz-DCM (data not shown). In series 3, we elected to study compounds that demonstrated the greatest cardioprotection in series 1 and 2. Heart weights, body weights, and heart weight to body weight ratios were similar to those in series 2 (data not shown). After 2 weeks of receiving Fz, cardiac dilatation was clearly evident. Heart weights of Fz-DCM animals were the largest of any group, similar to findings in series 1 and 2 (10.3±2 versus 6±1.5 g for Fz versus control, respectively; *P*<.05). Similar to series 2, heart volumes (eg, left ventricle balloon volumes measured at peak left ventricular developed pressure) were smaller for animals receiving propranolol plus Fz versus atenolol plus Fz and smaller for nifedipine plus Fz versus verapamil plus Fz (Table 2). Cardioprotective agents administered to normal birds did not affect gross morphology or whole heart contractile function (Table 2, Fig 4). We elected not to study atenolol alone after demonstrating that nifedipine, verapamil, and propranolol alone produced no significant difference in isolated muscle and whole heart Langendorff preparations. As a result, animals receiving only atenolol were not studied with Langendorff preparations.
Langendorff Preparations

In Langendorff-perfused hearts, there were no significant differences in spontaneous heart rate or coronary flow per gram among different treatment groups (Table 2). However, as demonstrated in Fig 4, there was reduced peak developed pressure in Fz-DCM animals. Animals receiving Fz plus a cardioactive agent developed pressures similar to control pressures (Fig 4). Administration of the β-receptor antagonists and Ca<sup>2+</sup> channel antagonists alone to normal animals did not affect peak developed pressures (Fig 4). Fz-DCM animals demonstrated a reduced +dP/dt and −dP/dt. Hearts from animals treated with propranolol, atenolol, and nifedipine plus Fz demonstrated +dP/dt and −dP/dt values similar to control values. Hearts from animals treated with verapamil plus Fz and with verapamil alone demonstrated a significant increase in both +dP/dt and −dP/dt (Table 2). Peak developed pressures and end-diasstolic pressures were similar for all treatment groups, as demonstrated in Fig 4.

Isolated Muscle Studies

Peak twitch force corrected for cross-sectional area for animals receiving Fz plus a cardioactive agent was similar at 30°C at a 1-Hz stimulation rate (Fz plus propranolol, 2.3±1.0 g/mm<sup>2</sup>, n=11; Fz plus atenolol, 2.6±0.5 g/mm<sup>2</sup>, n=9; Fz plus nifedipine, 2.8±0.9 g/mm<sup>2</sup>, n=19; Fz plus verapamil, 2.9±0.8 g/mm<sup>2</sup>, n=15) (P>.05). Peak twitch force was 1.8±0.4 (n=13) and 2.1±0.7 (n=6) g/mm<sup>2</sup> for control and Fz-DCM, respectively. Because all groups demonstrated similar peak twitch force, we normalized contractile response to increasing [Ca<sup>2+</sup>], to the maximal Ca<sup>2+</sup> response. This permitted us to assess the potency of varying [Ca<sup>2+</sup>], on contractile response. Ca<sup>2+</sup> responsiveness was not different between control (n=13), Fz-DCM (n=6), propranolol plus Fz-treated (n=11), atenolol plus Fz-treated (n=9), nifedipine plus Fz-treated (n=19), or verapamil plus Fz-treated (n=15) birds after 1 or 2 weeks of treatment (P>.05) (Fig 5).

We were surprised to find similar peak twitch forces for the Fz-DCM and control groups. We hypothesized that our experimental finding might be frequency de-
We group). Therefore, we studied this phenomenon in Fz-DCM, Fz plus nifedipine–treated, and Fz plus propranolol–treated muscles stimulated at closer to physiological heart rates at 37°C (Fig 6). As before, at 37°C and a 1-Hz stimulation rate, we found peak twitch forces to be similar. However, at higher frequencies (>1 Hz), Fz-DCM muscles generated significantly less peak twitch force (1.5±0.4 versus 4±0.5 g/mm² for Fz-DCM and control, respectively, at 2 Hz; n=5 muscles per group). We addressed the question as to whether the blunted/negative force-interval relation seen in Fz-DCM was altered by treatment with propranolol or nifedipine, agents found to be the most cardioprotective. As shown in Figs 6B and 6C, the force-interval relation was similar in isolated muscles from the hearts of control animals not receiving Fz and hearts of Fz plus propranolol–treated (n=6) or Fz plus nifedipine–treated (n=10) animals. The negative force-interval relation seen in myopathic (Fz-DCM) animals was not present.

To investigate potential mechanisms for the improved contractile performance, we measured CK activity and ATP synthesis via the CK system, which is 10 times faster than the ATP synthesis via oxidative phosphorylation. Thus, the CK system is important for energy reserve and is needed to support contractile performance.18 It has previously been demonstrated by us19 as well as others17 that CK activity is significantly diminished in Fz-DCM. CK activity was 6.4±1.2 IU/mg protein (n=7) in control animals and 4.2±0.5 IU/mg protein (n=4) in Fz-DCM animals (P=.007). In both the propranolol–treated Fz-DCM group (6.53±0.62 IU/mg protein, n=5) and the nifedipine–treated Fz-DCM group (5.97±0.74 IU/mg protein, n=5), CK ac-

![Graph showing contractile responsiveness in isolated muscle preparations expressed as percent of maximal Ca²⁺ response after 2 weeks of treatment. Fz indicates furazolidone–induced dilated cardiomyopathy; Nif, nifedipine; Ver, verapamil; Aten, atenolol; and Prop, propranolol. Numbers of muscle preparations per group are as follows: control, 13; Fz, 6; Nif plus Fz, 19; Aten plus Fz, 9; Ver plus Fz, 15; and Prop plus Fz, 11. Standard deviation bars have been omitted for clarity of presentation. There were no differences between the groups. Nif alone has been included to document reproducibility of experimental results as reported in a previous study.](http://circres.ahajournals.org/)

![Graph showing force-frequency relation in control group and group with furazolidone–induced cardiomyopathy (Fz) (n=5 per group) at 37°C ([Ca²⁺]o, 2.0 mmol/L). B and C, Graphs show force-frequency relation in Fz group receiving propranolol (Prop, n=6 per group, B) and nifedipine (Nifed, n=10 per group, C) at 37°C ([Ca²⁺]o, 2.0 mmol/L).](http://circres.ahajournals.org/)
tivity was similar to values obtained in control animals. Compared with untreated Fz-DCM animals, these values were significantly higher ($P \leq .005$). In an animal that demonstrated moderate to poor cardioprotection (eg, dilatation and wall thinning) with nifedipine, the CK activity measured from three areas of the left ventricle was 4.72±0.36 IU/mg protein ($P=0.2$ compared with Fz-DCM). After pooling values from all the hearts, a negative correlation was found between CK activity and heart volume ($r=-0.8$). These data suggest that CK activity corresponds to the greater cardioprotection seen with propranolol versus nifedipine.

Capacity for ATP synthesis by oxidative phosphorylation was estimated by measurement of mitochondrial F$_1$-ATPase activity, which is attributed to the synthase working in reverse in vitro.14 F$_1$-ATPase activity was 0.33±0.03 IU/mg protein ($n=8$) in control animals. It was 15% lower for Fz-DCM animals (0.28±0.02 IU/mg protein, $n=5$). Propranolol and nifedipine prevented the Fz-DCM-induced decrease in F$_1$-ATPase activity. Propranolol-treated animals had 21% higher F$_1$-ATPase activity (0.40±0.03 IU/mg protein) than did nifedipine-treated (0.33±0.02 IU/mg protein) and control animals.

**Twitch Time Course**

It has been previously demonstrated that increasing [Ca$^{2+}$], does not significantly affect the twitch time course in nonfailing myocardium.23 Therefore, we pooled time courses at all levels of [Ca$^{2+}$] for each group. There were no differences in time to peak tension or 50% relaxation from peak tension for any of the treatment groups except the Fz-DCM and verapamil plus Fz-treated groups. In muscles studied at 30°C, the time to peak tension and the time to 50% relaxation were as follows (milliseconds): control, 194±9 and 116±20 (n=13); Fz-DCM, 223±7 ($P<.05$ compared with control) and 127±10 (n=6); nifedipine plus Fz, 212±30 and 116±26 (n=19); verapamil plus Fz, 214±26 ($P<.05$ compared with control) and 121±18 (n=15); atenolol plus Fz, 191±26 and 119±15 (n=9); and propranolol plus Fz, 194±15 and 114±11 (n=11). Fz-DCM animals and verapamil plus Fz-treated animals demonstrated longer time to peak tensions compared with control animals. We have previously demonstrated that time to 80% relaxation is prolonged in Fz-DCM.24

**SR Ca$^{2+}$-ATPase and Ca$^{2+}$ Cycling**

SR Ca$^{2+}$-ATPase activity was 16.0±1 versus 16.1±2 mU/g tissue for control and Fz-DCM groups, respectively. Fz-DCM produced no effect on the net Ca$^{2+}$-sequestration activity of the SR. The net Ca$^{2+}$-sequestration rates were 41.13±6.65 nmol/L per second in control animals compared with 33.83±4.74 nmol/L per second in Fz-DCM animals. Ca$^{2+}$ concentration of the homogenates was 1460±204 nmol/L for control animals and 1471±97 nmol/L for Fz-DCM animals ($P<.1$).

Despite the lack of effect on net Ca$^{2+}$-uptake activity, Fz produced substantial downregulation in Ca$^{2+}$-pumping activity (28% inhibited) and in Ca$^{2+}$-release channel activity (39% inhibited), the two opposing activities that determine net Ca$^{2+}$ uptake. The maximal Ca$^{2+}$-pumping activity of the SR was 58.23±8.84 nmol/L per second in control animals and 41.99±4.57 nmol/L per second in Fz-DCM animals, whereas the maximal Ca$^{2+}$-release channel activity was 31.03±4.84 and 18.84±3.93 nmol/L per second in control and Fz-DCM animals, respectively. As a result of the decreases in these two activities, the total Ca$^{2+}$ cycling was reduced by 29%. The total Ca$^{2+}$-cycling activity was 90.07±11.93 nmol/L per second for control animals and 60.83±8.26 nmol/L per second for Fz-DCM animals. The greater downregulation of the Ca$^{2+}$-release channel relative to the Ca$^{2+}$ pump resulted in the preservation of the net Ca$^{2+}$ sequestration of the SR.

Compared with the control group, activities were similar for Ca$^{2+}$ pump, Ca$^{2+}$ channel, and total Ca$^{2+}$ cycling in the propranolol-treated (58.91±9.71, 28.13±8.95, 87.04±17.91 nmol/L per second, respectively; $n=5$) and nifedipine-treated (56.56±12.59, 34.43±9.46, and 90.99±21.9 nmol/L per second, respectively; $n=5$) groups. Also compared with the control group, Ca$^{2+}$ concentrations of homogenates and extent of closure of the Ca$^{2+}$ release channel were similar for the propranolol-treated (1470±380 nmol/L and 61.4±32.0%) and nifedipine-treated (1515±553 nmol/L and 34.43±9.46%) groups. However, net Ca$^{2+}$ sequestration for propranolol-treated animals (46.54±9.98 nmol/L per second) was 35% greater than for Fz-DCM animals and 40% greater than for nifedipine-treated animals (32.51±6.86 nmol/L per second). These differences in net activity could be attributed to the Ca$^{2+}$-release channel in the untreated state being 48% more closed for propranolol-treated animals than for all other animals (41.45±14.08% by two tailed $t$-test).

**Discussion**

**Animal Models: Comparison With Human Myocardium**

Earlier work from our laboratory has shown many similarities between normal avian and mammalian myocardium with respect to myosin and myofibrillar ATPase activity, the $\beta$-receptor--adenyl cyclase transmembrane signaling, Ca$^{2+}$ channel number, myofilament Ca$^{2+}$ responsiveness, CK, citrate synthase and lactate dehydrogenase activities, norepinephrine content, and myocardial creatine content.1 Our studies of Fz-DCM show similarities in contractile function, force-pCa relations, reduced myofilibr protein content and myofibrill ATPase activity, reduced CK activity and creatine content, slowed crossbridge cycling rates, and troponin T and troponin I isofrom switching as reported in failing human myocardium, therefore making this model appropriate for the study of the pathogenesis and pathophysiology of cardiomyopathy as well as the potential cardioprotective effects of therapeutic interventions.1,18,24-27 Because of the similarities in pathophysiology, our findings are likely to relate to human myocardium.

The present study demonstrates differences between this model and the Syrian hamster model of cardiomyopathy.10 In our model, both propranolol and nifedipine are cardioprotective, whereas they have not proven to be effective in the Syrian hamster model of cardiomyopathy. Furthermore, our study demonstrates that not only the choice of an agent but also the dose is important in determining therapeutic efficacy and that subtherapeutic dosages of agents may have direct cardiac effects.1,28 Our results indicate that treatment with
verapamil or the \(\alpha_1\)-adrenergic receptor antagonist phenoxybenzamine is not as beneficial in preventing the development of Fz-DCM as is propranolol or nifedipine. Conversely, treatment with verapamil and the \(\alpha_1\)-adrenergic receptor antagonist prazosin was effective in the Syrian hamster model of cardiomyopathy.29 Verapamil acts as a competitive inhibitor of myocardial \(\alpha_1\)-adrenergic receptors, which may explain why verapamil, like prazosin, is cardioprotective in the Syrian hamster model. This would also explain why nifedipine, which is not a competitive inhibitor of \(\alpha_1\)-adrenergic receptors, has not proven to be beneficial in this model.31 These dissimilar findings suggest that the pathogenesis of cardiomyopathy in the Syrian hamster differs from that of the turkey poult.

The exact mechanism by which Fz, a nitrofuran antifungal, acts to produce dilated cardiomyopathy in avian species is unknown. Fz can act as a potent monoamine oxidase inhibitor;32 however, there are several lines of evidence suggesting that this is not the case. First, Powers et al33 have shown that monoamine oxidase inhibition produced by tranylcypromine, a chemically unrelated compound, does not produce cardiomyopathy in turkeys. Second, histopathologic findings associated with catecholamine-induced cardiomyopathy are not seen in Fz-DCM hearts.25-33,34 Preliminary data from our laboratory show that animals receiving Fz experience neither hypertension nor tachycardia during the development of cardiomyopathy, which argues against excess catecholamine stimulation induced by Fz ingestion. In addition, animals fed Fz that do not develop overt heart failure have normal norepinephrine turnover rates and content.24

Pathophysiology of Fz-DCM

The present study demonstrates that, similar to changes in cardiac chamber dimension, global cardiac function was depressed. The decreased contractile performance demonstrated in Langendorff-perfused hearts from Fz-DCM animals was not due to hypoxia, because the hearts received a flow per gram similar to that in control hearts, arguing strongly that the decreased contractile performance is a real observation. As seen in failing human myocardium, peak developed pressures, as well as the rate of pressure development and decline, were reduced in myopathic animals. Because the peak left ventricular systolic pressure was proportionately lower in Fz-DCM hearts versus control hearts (ie, the ratio of \(-\mathrm{dP}/\mathrm{dt}\) to left ventricular systolic pressure is similar; see Fig 4), one would expect the time to 50% relaxation to be unchanged, which is consistent with our findings of similar times to 50% relaxation in Fz-DCM and control isolated muscle studies. It is interesting that all agents (ie, \(\beta\)-receptor antagonists and \(\mathrm{Ca}^{2+}\) channel antagonists) that were beneficial in preventing the development of the dilated cardiomyopathy also prevented the changes in peak developed pressure, \(+\mathrm{dP}/\mathrm{dt}\), and \(-\mathrm{dP}/\mathrm{dt}\). It is also interesting that hearts from verapamil plus Fz–treated animals demonstrated larger cavity dimensions yet, at the same time, displayed normal contractile performance. These data may indicate that with verapamil treatment the heart moves up on the Starling curve, thereby preserving contractile function. Although there was no significant difference between left ventricular end-diastolic pressure in Fz-DCM and control hearts, the left ventricular end-diastolic wall stress is probably greater in the Fz-DCM group despite the lower left ventricular end-diastolic pressure when one considers the Laplace relation given the fivefold higher radius to thickness ratio of the Fz-DCM versus control hearts (see Fig 2). This is consistent with the known higher diastolic wall stress associated with dilated cardiomyopathy.

As previously demonstrated for failing human myocardium, peak developed twitch force and muscle \(\mathrm{Ca}^{2+}\) responsiveness were similar for muscles from normal and failing hearts at relatively slow stimulation rates.35 These data indicate that hearts from animals with heart failure have the potential to generate normal levels of force, as has been demonstrated in human heart failure. However, this is unlike the guinea pig model of hypertrophy and failure and the pacing-induced heart failure model in dogs, which demonstrate a markedly reduced inotropic response to \(\mathrm{Ca}^{2+}\) stimulation.35,36 These data suggest that this model of dilated cardiomyopathy may prove useful in the study of the pathogenesis of dilated cardiomyopathy and the effects of therapeutic agents.

The observation that twitch time courses and \(\mathrm{dP}/\mathrm{dt}\) were normalized with pharmacologic intervention suggests that changes in SR \(\mathrm{Ca}^{2+}\) mobilization seen in heart failure were prevented and supports a causative role for abnormal \(\mathrm{Ca}^{2+}\) handling in this model of cardiomyopathy similar to what has been reported in humans.37 Furthermore, at more physiological heart rates, there was a significantly reduced peak twitch force similar to findings in human myocardium38-40 and clinically seen in human heart failure and our Langendorff heart preparations. These experiments emphasize the need to perform experiments at closer to physiological heart rates.

The frequency of stimulation for isolated muscles demonstrated a difference at 1.5 to 2 Hz. Normal turkey pouls of a similar age have resting heart rates of \(\approx 170\) beats per minute. The observation that muscles from animals receiving agents found to be cardioprotective demonstrated a positive force-frequency relation at physiological heart rates indicates that primary subcellular defects of Fz-DCM can be prevented. In the present study, we have shown that subcellular changes in SR function and \(\mathrm{Ca}^{2+}\) mobilization as well as myocardial energetics were indeed prevented by these agents.

The lack of change in net \(\mathrm{Ca}^{2+}\) sequestration activity and SR \(\mathrm{Ca}^{2+}\)-ATPase activity, which appears not to be downregulated, is similar to reports in failing human myocardium.41,42 That \(\mathrm{Ca}^{2+}\)-pumping activity was decreased despite the lack of change in \(\mathrm{Ca}^{2+}\)-ATPase activity confirms a previous report of uncoupling of these activities in Fz-DCM.43 The asymmetric downregulation of the \(\mathrm{Ca}^{2+}\) pump and \(\mathrm{Ca}^{2+}\)-release channel activities and the reduction in total \(\mathrm{Ca}^{2+}\) cycling observed in Fz-DCM are similar to those reported for several canine models of naturally occurring idiopathic dilated cardiomyopathy and experimentally induced congestive heart failure.12 Our observations might also reflect leakage from the SR. The decrease in \(\mathrm{Ca}^{2+}\)-pumping activity would support our findings of a slower rate of contraction and relaxation (ie, \(+\mathrm{dP}/\mathrm{dt}\) and \(-\mathrm{dP}/\mathrm{dt}\)). \(\mathrm{Ca}^{2+}\) metabolism and muscle energetics are perturbed in this model, similar to reports in failing human myocardium.25,44 In several studies, authors have
speculated that the asymmetric downregulation of the Ca\(^{2+}\) cycle is an adaptive strategy of the myocardium to reduce the energy required for Ca\(^{2+}\) cycling by improving the efficiency of Ca\(^{2+}\) uptake.\(^{12,14,22}\) In support of this idea, we have found that markers of energy supply, eg, CK and F1-ATPase activity, are significantly reduced in Fz-DCM.\(^{18}\) The creatine phosphate concentration and forward CK reaction velocity measured using \(^{31}\)P saturation transfer are significantly lower in Fz-DCM hearts.\(^{18}\) Thus, CK activity, creatine phosphate and ATP concentrations, and forward CK reaction velocity decrease with decreased cardiac performance in Fz-DCM. Unlike in normal myocardium, where energy flux increases with heart rate, in Fz-DCM hearts energy flux (ie, forward reaction velocity of CK) is rate independent.\(^{18}\) Taken together, these results support the hypothesis that abnormal Ca\(^{2+}\) mobilization, a decrease in energy reserve via the CK system, and a decrease in energy supply by oxidative phosphorylation contribute to the decreased pump function in Fz-DCM.

**Mechanisms of Beneficial Effects Seen With Cardioprotective Agents**

**Antioxidant properties.** The cardioprotection afforded by treatment with nifedipine or propranolol in Fz-DCM could possibly be related to the antihypertensive effects of these agents. However, the fact that verapamil and phenoxycbenzamine, which are also vasodilators, were not as effective suggests that other mechanisms are involved. Furthermore, doses of agents were studied that did not affect heart rate or blood pressure.\(^1\) One possibility is that exposure to Fz results in the production of free radicals, which can cause membrane damage, and that propranolol and nifedipine, which are effective antioxidants, exert a cardioprotective effect by acting at the sarcolemma to protect against free-radical injury.\(^{44}\) Studies with vitamin C, a potent antioxidant, suggest that antioxidant properties may in part contribute to the cardioprotection.

**Ca\(^{2+}\) entry via Ca\(^{2+}\) channels and \(\beta\)-adrenergic receptor modulation.** The primary defect in Fz-DCM may involve altered Ca\(^{2+}\) influx across the cardiac myocyte membrane, arising as a direct result of changes in Ca\(^{2+}\) channel structure, number, and/or function. The differences in effectiveness observed for different classes of Ca\(^{2+}\) channel antagonists in this study could be a result of their action at different sites along the Ca\(^{2+}\) channel, could reflect the effects on different L-type Ca\(^{2+}\) channel subtypes recently reported, or could be a result of non-Ca\(^{2+}\) channel-related effects. Modulation of sarcolemmal Ca\(^{2+}\) influx through alterations in \(\beta\)-adrenergic receptor activity may also contribute to the development of Fz-DCM. There is ample experimental evidence demonstrating that both short-term\(^{46}\) and chronic\(^{47,48}\) alterations in \(\beta\)-adrenergic receptor activity can modulate Ca\(^{2+}\) channel activity. Investigators working on an inbred avian model of congestive cardiomyopathy have proposed that chronic \(\beta\)-adrenergic receptor modulation of sarcolemmal Ca\(^{2+}\) influx is involved in the development of cardiomyopathy.\(^{49}\) We have demonstrated that propranolol at a dose of 3 mg/100 g body wt is therapeutic in a similar model of idiopathic dilated cardiomyopathy as well as Fz-DCM, resulting in a significant reduction in cardiac dilatation and improved contractile function.\(^{6,50}\)

The nonselective \(\beta\)-adrenergic receptor antagonist propranolol appeared to be more cardioprotective than the \(\beta_1\)-adrenergic receptor antagonist atenolol. Similar observations have been made by our laboratory when comparing the therapeutic effects of propranolol, atenolol, and metoprolol in Fz-DCM animals. Propranolol produced greater resolution of the cardiomyopathic dilatation than did atenolol or metoprolol (authors' unpublished data). It is probable that selective \(\beta_1\)-adrenergic receptor downregulation is not an important factor in the pathogenesis of cardiomyopathy. Rather, uncoupling of \(\beta_2\)-adrenergic receptors may be involved. In failing human myocardium, uncoupling of \(\beta_2\)-adrenergic receptors, which are important in maintaining myocardial contractility, occurs in addition to downregulation of the more numerous \(\beta_1\)-adrenergic receptors.\(^{51}\)

Recently, the nonselective \(\beta\)-receptor antagonist carvedilol has been shown to restore contractile function in the setting of heart failure in the absence of a change in \(\beta\)-receptor number.\(^{52}\) Preliminary data from our laboratory also suggest that, with the Fz-DCM model, there is near normalization of cardiac function, with only a <30% increase in the reduced \(\beta\)-receptor number seen with Fz-DCM (unpublished data), similar to studies in human patients.\(^{53}\) Another potential explanation for the differential effects of the two \(\beta\)-receptor antagonists investigated is that propranolol has a very high affinity for avian \(\beta_2\)-adrenergic receptors, whereas atenolol has only low affinity. However, in contradistinction to this suggestion is the lack of greater cardiac protection with higher concentrations of atenolol (series 2).

**Subcellular remodeling.** Animals receiving a cardioprotective agent plus Fz displayed normal contractile performance at both the whole heart and isolated muscle levels, showing that myocytes are the target cells for these interventions. Agents that were found to be cardioprotective (eg, atenolol, propranolol, nifedipine, and verapamil) for Fz-DCM animals also showed beneficial effects in normal animals, as assessed by changes in the composition of transmembrane signaling pathways and energy availability.\(^3\) Atenolol and propranolol both improved energy reserve, with propranolol having the greater effect in normal animals. Propranolol increased energy reserve, as demonstrated by increases in the tissue content of the guanidino substrate for CK (23%), CK activity (23%), lactate dehydrogenase (17%), and mitochondrial ATP synthetase (22%). Furthermore, compared with nifedipine-treated animals, Ca\(^{2+}\) channel activity of the propranolol-treated animals was 95% more inhibited, which resulted in 40% greater net Ca\(^{2+}\)-sequestration activity. This resulted in the net Ca\(^{2+}\) sequestration being enhanced and restored to normal values for the propranolol-treated animals. It has been reported and confirmed here that CK activity is reduced in Fz-DCM.\(^{15,37}\) Isolated muscles from hearts of animals receiving Fz plus propranolol or nifedipine demonstrated a normal force-interval relation. In these hearts, CK activity was similar to values measured in hearts from control animals not receiving any agent. F1-ATPase was 21% higher in hearts from animals treated with propranolol than in control hearts not receiving an agent. Of the number of agents studied, propranolol-treated animals demonstrated the greatest cardioprotection. There was a negative correlation between CK activity and heart size. Thus, these data
indicate that reversal of the negative force-frequency relation correlates with improvement in energy reserve.

Both propranolol and nifedipine treatment resulted in a normal force-interval relation. We could not determine whether aberrations in SR Ca\(^{2+}\) handling or myocardial energetics play a greater role or are directly responsible for the negative force-interval relation seen in Fz-DCM, because in this study both changed concurrently. Nevertheless, it is important to note that propranolol, which demonstrated the greatest cardioprotection, had a greater effect on myocardial energetics and SR Ca\(^{2+}\) handling.

Nifedipine and verapamil both increase the \(\beta\)-receptor adenylate cyclase activity in normal animals. In addition, verapamil increased maximal myofibrillar ATPase activity. In contradistinction, nifedipine was found to increase maximal Ca\(^{2+}\)-activated force in skinned fiber preparations. Agents found not to be cardioprotective, eg, digoxin or phenoxybenzamine, did not affect transmembrane signaling pathways or induce subcellular remodeling. Therefore, it is clear that the pharmacologic agents investigated affect signaling pathways in a manner conducive to the prevention of subcellular changes seen in this model of cardiomyopathy. Furthermore, as in human heart failure, there is a reduction of key enzymes involved in energy supply. Our observations suggest that changes in energy balance and SR Ca\(^{2+}\) handling may play a role in the negative force-frequency relation in this model as well as in human myocardium. Failing human myocardium also has a negative force-interval relation and a significant reduction in CK activity.

**Summary**

The results of the present study indicate that the Fz-DCM model of cardiomyopathy provides a useful model for studying the pathogenesis and pathophysiology of cardiomyopathy as well as the effects of cardioactive agents. Importantly, agents found to be cardioprotective prevented the development of derangements in Ca\(^{2+}\) mobilization, myocardial energetics, and contractile performance. Agents found to be the most cardioprotective induced subcellular remodeling in a manner conducive to the observed prevention of cardiac dilatation and wall thinning in the absence of hemodynamic effects. Further studies on muscle physiology and membrane receptor function in this model should improve our understanding of the pathogenesis and pathophysiology of dilated cardiomyopathy as well as mechanisms for therapeutic effects of cardioactive agents.

**Acknowledgments**

This study was supported in part by the Institute for the Study of Treatments for Cardiovascular Diseases, Cardiovascular Drug Development and Marketing Consultants, the John H. and Robnette T. Gwathmey Estate, National Heart, Lung, and Blood Institute Grants HL-39091 and HL-36797, Glaxo Inc, and the Upjohn Co (Dr Gwathmey); by National Institutes of Health Grant HL-38189 (Dr Ingwall); and by a grant from the Central Ohio Heart Chapter, Inc (Dr Hamlin). Dr Gwathmey is an Established Investigator of the American Heart Association. Kathi Thorp, Dr Ellen Weinberg, and Ilana Reis are thanked for technical assistance. Michael Lanner and Catherine Weeks of the Animal Research Facility of Beth Israel Hospital are thanked for their support throughout the housing of the animals. Drs James D. Marsh and Walter H. Abelmans are thanked for their helpful discussions. Dr Bernard Ransil is thanked for statistical consultation.

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Circ Res. 1993;73:1077-1089
doi: 10.1161/01.RES.73.6.1077

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

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