Increased Heart Rate Prevents the Isomyosin Shift After Cardiac Transplantation in the Rat

David L. Geenen, Ashwani Malhotra, Peter M. Buttrick, and James Scheuer

The heterotopically transplanted rat heart undergoes significant atrophy and a shift from V₁ to V₃ isomyosin. The purpose of this study was to pace the cardiac isograft and determine whether an increase in heart rate would attenuate the changes in cardiac mass and isoenzyme distribution. Nonpaced transplanted hearts were compared with hearts in which pacing was initiated at 7 Hz, 24 hours after transplantation, and continued for 7 days. There was a 29% decrease in myosin ATPase activity and a 22% decrease in α-myosin in the nonpaced isograft; both decreases were completely prevented by pacing. The decrease in cardiac mass was also significantly attenuated. Pacing did not alter intrinsic heart rate, systolic pressure, dP/dt, or norepinephrine concentration in the isograft. These results suggest that the adaptation in both cardiac mass and isoenzymes may be related to the rate or the rate-pressure product in the transplanted paced heart independent of left ventricular pressure, tissue catecholamines, or neural activity. (Circulation Research 1992;70:554–558)

KEY WORDS  •  hemodynamic load  •  heart rate  •  catecholamines  •  isoenzymes  •  myosin ATPase activity  •  cardiac mass

N eurohumoral and hemodynamic mechanisms have been implicated in mediating cardiac adaptations in the rat after physiological and pathological stress.1–4 Swimming and treadmill running induce elevated plasma levels of circulating norepinephrine and epinephrine,3,5 and myocardial tissue levels of norepinephrine are significantly increased with chronic swimming, commensurate with changes in isomyosin distribution and cardiac mass.2,3 Furthermore, systolic overload from renal hypertension and myocardial infarction is associated with changes in cardiac contractile proteins, morphology, and function and with diminished myocardial norepinephrine levels.6–8 All of these factors acting in concert make it difficult to dissociate the contribution of the sympathetic nervous system, circulating catecholamines, and hemodynamics from their individual effect on the myocardium. Previous studies suggest that, although increased sympathetic activation and endogenous catecholamines have been implicated as contributing factors in the cardiac hypertrophy process, muscle load is the primary determinant of the hypertrophic response and that the neurohumoral system does not play an important role in mediating that response.9,10 Several investigators have reported that chronotropic activity of the heart is responsible for the difference observed in the isomyosin distributions across species; these reports are based on studies of mammalian cardiac muscle1,11 and studies of the isomyosin composition of the rat cardiac isograft in which the intrinsic heart rate is decreased.12

The rat heterotopic cardiac isograft has recently been used to examine the role of hemodynamic unloading on cardiac biochemical adaptations in the absence of neural innervation.12–14 It has been demonstrated that marked atrophy and a shift from a predominant percentage of V₁ to V₃ myosin isoenzyme occur as early as 1 week after transplantation,15,16 whereas chronic swimming and thyroid hormone attenuate or prevent this shift from occurring.14,17

The purpose of this study was to increase the intrinsic heart rate of the rat cardiac isograft by pacing and to examine whether cardiac mass, hemodynamics, and contractile proteins were influenced by the change in heart rate. We hypothesized that, if heart rate was a significant contributor to an observed shift in cardiac myosin isoenzymes and overall cardiac work, pacing the denervated hearts higher than the intrinsic rates of the nonpaced isograft would attenuate both the V₁ to V₃ shift and the atrophy previously observed in this model. Alternatively, if denervation of the isograft was primarily responsible for muscle atrophy and the shift in myosin isoenzymes, increasing cardiac frequency by pacing would not appreciably alter cardiac mass and contractile proteins.

Materials and Methods

Inbred female Fischer 344 rats weighing 150–175 g were obtained at 10 weeks of age from Charles River Laboratories, Inc., Raleigh, N.C., and used in all the experiments. Two groups of rats were studied: 1) rats with cardiac isografts in which hearts were paced continuously for 7 days after transplantation and 2) rats

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with cardiac isografts in which pacing wires were implanted but not connected to a stimulator.

Infrarenal cardiac transplantation was performed as previously reported\textsuperscript{14} and modified.\textsuperscript{18} Briefly, the rats were pretreated with butorphanol (0.2 mg/kg s.c., Stadol, Bristol Laboratories, Syracuse, N.Y.) and anesthetized with 4% chloral hydrate (0.8 mg/100 g i.p.). The aortic and pulmonary trunks of the donor heart were anastomosed end to side to the abdominal artery and the inferior vena cava of the recipient, respectively. The isograft began beating immediately after reperfusion.

In rats from both groups, two 36-gauge biomedical wires (No. AS633, Conner Wire Co., Chatsworth, Calif.) were sutured to the right ventricular epicardial surface of the isograft, passed through the abdominal wall, and tunneled subcutaneously to the dorsal surface of the neck. The abdominal muscle layer was closed with 5-0 chromic gut suture, and the skin was closed with wound clips. A third wire was sutured subcutaneously to the back of the neck to monitor the electrocardiogram of the native heart, and the rat was fitted with a rodent jacket and a spring tether to protect the pacing wires (Alice King Chatham Medical Arts, Hawthorne, Calif.). The rats were able to ambulate normally with the harness system and had access to water and chow ad libitum. To eliminate intragroup and intergroup variability in baseline body and heart mass, surgery was performed on rats from the same shipment and on one rat from each group on the same day. Before surgery, body mass was matched between donor and recipient as well as between pacing and nonpacing groups.

The rats to be paced were allowed to recover from surgery for 24 hours, after which the pacing wires were connected in series to a stimulus isolation unit (model SIU5, Grass Instrument Co., Quincy, Mass.) and stimulator (model S44, Grass Instrument), and square-wave pulses at 7 Hz and of 5-msec duration were delivered continuously. The threshold level of pacing was determined, and the voltage was adjusted to 10% above this threshold level. The hearts were palpated twice daily with the stimulator on and off to determine whether the isograft was being adequately paced. The pacing wires were temporarily removed from the stimulator twice daily to record electrocardiograms from the native and transplanted hearts in the paced group. Electrocardiograms were also obtained in the nonpaced group at the same time intervals.

In two rats from both groups, the cardiac isograft was instrumented with an ultraminiature pressure transducer (model SPR-407, Millar Instruments, Inc., Houston, Tex.). The pressure transducer tip was passed through an incision in the left atrium and secured with 4-0 silk at the base of the atrial appendage before the vascular anastomoses. The transducer wire was passed through the abdominal wall and tunneled subcutaneously with the pacing wires as previously described. Left ventricular pressure and $\pm $dP/dt of the isograft were recorded daily in the awake rat. Seven days after transplantation, rats in the nonpaced and paced groups were anesthetized with methoxyflurane (metofane, Pitman-Moore, Mundelein, Ill.) and killed. Both the native hearts and the cardiac isograft were removed, and the atria, pulmonary artery, and aorta were trimmed off. The right ventricular free wall was also dissected from the left ventricle and septum. The left ventricle was divided into two pieces for subsequent tissue catecholamine and myosin isoenzyme and ATPase analysis. Cardiac tissue samples for norepinephrine and epinephrine analyses were homogenized in 2.5 ml of 0.1 M HClO$_4$ plus 2.5 mg Na$_2$S$_2$O$_7$ and centrifuged (5,000g) for 30 minutes. The supernatant was frozen for subsequent high-performance liquid chromatography–electrochemical analysis.\textsuperscript{3}

ATP, dithiothreitol, EDTA, and proteolytic enzyme inhibitor phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co., St. Louis, Mo. Hearts were stored at $-70^\circ$C in 50% glycerol containing (mM) KCl 30, K$_2$PO$_4$ 10 (pH 7.0), dithiothreitol 2, and PMSF 0.2 before the preparation of myofibrillar extracts. Cardiac myofibrils were isolated and purified with Triton X-100 by a modified technique.\textsuperscript{19} In all myofibrillar preparations, 1 mM dithiothreitol and 0.1 mM PMSF were used throughout the purification procedure. Briefly, ventricles were minced and homogenized in buffer A, which consisted of 0.05 M KCl, 0.01 M KPO$_4$ (pH 7.0), 2 mM MgCl$_2$, 1 mM dithiothreitol, and 0.1 mM PMSF. The homogenate was washed successively with buffer A plus 2 mM EGTA (pH 7.0) and finally with buffer A containing 0.1% Triton X-100. In the final step, MgCl$_2$ was omitted for Ca$^{2+}$-ATPase measurements. To check the purity of the myofibrils, the samples were run on a sodium dodecyl sulfate gradient (5–16.5%) slab gel electrophoresis in a Tris-glycine buffer system. No evidence of proteolytic breakdown was detected in these purified myofibrillar preparations. Cardiac myosin ATPase activity in myofibrils was measured in high ionic strength under dissociating conditions. Calcium-dependent myosin ATPase was assayed at 30°C in 0.3 M KCl, 50 mM Tris chloride (pH 7.6), 10 mM CaCl$_2$, 5 mM ATP, and 0.2 mg/ml cardiac muscle protein. The reaction was initiated by the addition of substrate and terminated after 10 minutes with 1.0 ml cold 10% trichloroacetic acid. Inorganic phosphate was determined as previously published.\textsuperscript{20} Results were expressed as micromoles of inorganic phosphate per milligram protein per minute. Protein concentration was determined by the biuret technique.

Analysis of myosin isoenzymes in purified myofibrils was performed by polyacrylamide gel electrophoresis using nondissociating conditions at 2°C in a Pharmacia apparatus (GE model 2/4) as described previously.\textsuperscript{21} The running buffer contained 20 mM Na$_2$P$_2$O$_7$ (pH 8.6), 10% glycerol, 1 mM EDTA, and 0.01% (vol/vol) $\beta$-mercaptoethanol. Cylindrical 4% polyacrylamide gels (60×6 mm) were prepared with acrylamide and N,N'$'$-methylene-bis-acrylamide (30:0.8). Approximately 5–6 $\mu$g crude myosin was layered on each gel and run at a constant voltage gradient of 14 V/cm for 20–22 hours. The gels were stained and destained, and their densitometric scans were integrated (model 3390 A, Hewlett-Packard Co., Palo Alto, Calif.). The relative estimate of each isoenzyme was calculated from the area under the peak height. For the calculation of $\alpha$-myosin, the V$_2$ isoenzyme was assumed to be equally distributed between V$_1$ and V$_1$, myosin isoenzyme. Analyses of cardiac mass, catecholamines, and biochemical data were performed using a one-way analysis of variance. Multiple comparisons between the four groups of hearts were performed with the Newman-Keuls test.\textsuperscript{22} Differences between groups were consid-
Heart rate was significantly greater than the mass of their nonpaced cohorts.

A representative tracing of left ventricular pressure and dP/dt of a paced cardiac isograft in an awake rat 3 days after transplantation is shown in Figure 2. The wires were briefly disconnected from the stimulator to obtain hemodynamic measurements during intrinsic rate and reconnected to obtain the same measurements during pacing. Although the heart was paced at a level 37% greater than that of the intrinsic rate, systolic pressure and its first derivative were unaltered. In separate experiments, we also observed that pacing the transplanted heart did not affect systolic pressure or heart rate in the recipient rat (authors’ unpublished observations).

Cardiac tissue catecholamine concentrations were normal and equivalent in the native hearts of the paced and nonpaced groups (Table 2). Norepinephrine levels in the cardiac isografts, as expected, were significantly depressed independent of continuous pacing. Epinephrine levels, unlike the neurotransmitter norepinephrine, reflect endogenous adrenal activity and did not significantly differ between native and transplanted hearts or between paced and nonpaced hearts.

Myosin ATPase activity was significantly depressed in the nonpaced isograft 7 days after transplantation (Table 3). Continuous pacing of the isograft completely prevented this change, and myosin ATPase activity was identical in the native and transplanted hearts of the paced rats. The isomyosin distribution displayed a similar response to pacing (Table 3). The percentage of V, myosin, which reflects the increased myosin ATPase activity, was significantly depressed by day 7 after surgery. Pacing resulted in an isoenzyme distribution that was similar in the isograft and native heart and did not differ from the native hearts of the nonpaced group.

### Table 2. Left Ventricular Tissue Catecholamine Levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpaced</th>
<th>Transplant</th>
<th>Nonpaced</th>
<th>Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>1.341±75</td>
<td>160±24*</td>
<td>1.426±104</td>
<td>174±22*</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>92±12</td>
<td>92±20</td>
<td>101±19</td>
<td>81±23</td>
</tr>
</tbody>
</table>

Values are mean±SEM of six rat hearts in each group. g, Gram of total heart mass.

*\( p \leq 0.05 \) vs. corresponding value for native heart.
**Table 3. Myosin Isoenzyme and ATPase Activity**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpaced</th>
<th>Paced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin ATPase (μM P, mg/min)</td>
<td>0.51±0.02</td>
<td>0.36±0.02*</td>
</tr>
<tr>
<td>α-Myosin isoenzyme (%)</td>
<td>67±4</td>
<td>52±3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of six hearts in each group. P, inorganic phosphate.

*p<0.05 vs. corresponding value for native heart; †p<0.05 vs. corresponding value for nonpaced heart.

**Discussion**

The major finding in the present study is that elevated heart rate is sufficient to attenuate a decrease in myocardial mass and prevent the shift in α-myosin previously observed in the transplanted rat heart.12,14,16,17 Our data would suggest that attenuation of cardiac mass in the paced isograft is not attributable to increased intraventricular pressure or muscle tension, since peak systolic and end-diastolic pressure were unaltered compared with the same heart when pacing was interrupted. Since heart rate was continuously elevated in the paced isograft while pressure was held constant, the total amount of tension per unit time was increased compared with the nonpaced isograft, and this may have contributed to the attenuation in cardiac mass with pacing. Other investigators have demonstrated that when the rate–pressure product was increased in the transplanted heart by implanting an aortic stent, left ventricular atrophy was significantly attenuated compared with the transplanted heart without the stent.24 However, increased muscle tension would probably not explain the prevention of the shift from V1 to V3, since interventions resulting in increased wall stress have demonstrated a predominance of the V3 isoenzyme.25,26

The rate of cardiac muscle atrophy occurs rapidly within the first week after transplantation.15 Although evidence suggests that protein synthetic capacity is reduced27 and that degradation, measured by lysosomal activity, may be increased,28 the proportion that each of these components contributes to protein turnover within the transplanted heart has not been elucidated. In the present study, ventricular pacing may have decreased protein degradation and/or increased protein synthesis, but to what extent pacing affects protein turnover in this rapid phase of muscle atrophy remains to be determined.

Recent studies of isolated neonatal cardiac myocytes implicated contractile activity and catecholamines as primary determinants of increased protein synthesis and myocyte size,29,30 whereas experiments using adult cardiocytes suggests that loading of the muscle cell mediates and may be directly related to alterations in cellular growth.10 In the intact heart, the interplay between neural activity and hemodynamic load complicates the dissociation of their independent contributions toward muscle growth. This dilemma has been elegantly addressed in studies of unloaded cat papillary muscles in vivo.9,31 In 1982, Cooper and Tomasek31 demonstrated that neural innervation and catecholamines do not mediate the hypertrophic response but that increased load may induce a local response; this response is possibly via sodium entry through stretch-dependent channels,32 which triggers cellular growth. The data from the present study would confirm and extend this hypothesis, since adaptations in muscle mass were achieved by increasing heart rate while systolic pressure and dP/dt were unchanged despite complete denervation and a virtual loss of normal cardiac tissue norepinephrine concentration.

Neither plasma catecholamines nor thyroid hormone alone is sufficient to affect cardiac muscle atrophy after transplantation.14,16,17 We have previously demonstrated that, despite increases in circulating norepinephrine and epinephrine associated with swimming,3 the atrophic response of the transplanted heart in rats exposed to 8 weeks of chronic swimming was not significantly different from transplanted hearts in sedentary controls even though native hearts of the swimming rats displayed significant hypertrophy.14

Although thyroid hormone promotes the V1 to V3 shift in cardiac isomyosin observed after transplantation, it does not attenuate cardiac atrophy.16,17 One possible reason for this failure to affect muscle mass may have been the inability of thyroid hormone to elicit a significant hemodynamic response in the transplanted heart. Heart rates in the studies by Korpeck et al.16 in 1987 and Klein and Hong17 in 1986 were 10–20% lower than the heart rates of the paced transplants in the present study. In addition, the effect of thyroid hormone on ventricular pressure in the transplanted heart was not determined.

Prevention of the V1 to V3 isoenzyme shift observed with pacing is remarkable and is the first instance, to our knowledge, in which direct evidence for heart rate–mediated control of isomyosin expression in cardiac muscle has been reported. Continuous low-frequency pacing in adult mammalian fast-twitch skeletal muscle induces a range of metabolic and biochemical adaptations characteristic of a slow-twitch muscle, such as increased oxidative capacity and shift in the myosin isoenzyme distribution.33 These adaptations have been analyzed at the transcriptional level, demonstrating that during continuous stimulation mRNA coding for slow myosin is increased and coding for fast myosin is depressed.34 Low-frequency pacing of the dog diaphragm, using a respiratory on–off cycle for 24–28 days, converted the mixed population of type I and II fibers to predominantly type I or slow myosin.35

Comparative studies11 of a spectrum of mammals with different resting heart rates have led to the hypothesis that heart rate may play a primary role in determining the isomyosin expression in cardiac muscle. Pacing studies of swine in which heart rates were elevated 50% above resting levels for 5–6 weeks demonstrated significantly increased sarcoplasmic reticular Ca2+–ATPase activity but no change in myosin ATPase activity or isomyosin expression.11,36 This generated the hypothesis that a certain threshold of cardiac contraction frequency exists at which a qualitative switch in
myosin isoenzyme expression occurs. Data from the present study support this hypothesis, since denervated hearts paced at 420 beats per minute exhibited a similar isomyosin distribution as the native hearts, while the percentage of V1 isoenzyme and myosin ATPase activity decreased in the nonpaced isografts relative to their native controls. The threshold heart rate level at which the isomyosin profile changes is probably higher than that of 300 beats per minute, as previously suggested, since the intrinsic rates of the nonpaced isografts in the present study maintained this level and still demonstrated a shift in the isoenzyme distribution.

In conclusion, increasing the frequency of contraction of the rat cardiac isograft after transplantation attenuates the atrophy observed in the nonpaced heart. This adaptation may be a work-related phenomenon resulting from the increased rate induced in the paced transplanted heart. Other factors such as circulating hormones, while not primary mediators, may play an important role in combination with hemodynamic load in maintaining and altering cardiac mass. A shift in the isomyosin distribution and decrease in ATPase activity of the nonpaced isograft is prevented by continuous pacing of the transplanted heart. This adaptation cannot be attributed to increased levels of tissue norepinephrine or neural activity. It is likely that the increased rate, independent of other neurohumoral and hemodynamic factors, is a trigger in the synthesis and/or degradation of the α-myosin heavy chain protein of the isograft.

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