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# Myocardial Gene Transfer and Overexpression of $\beta_2$ -Adrenergic Receptors Potentiates the Functional Recovery of Unloaded Failing Hearts

Hendrik T. Tevæarai, MD; Andrea D. Eckhart, PhD; G. Brant Walton, BS; Janelle R. Keys, PhD; Katrina Wilson, BS; Walter J. Koch, PhD

**Background**—Mechanical assistance of the failing left ventricle (LV) can lead to functional recovery after a period of unloading, including restoration of  $\beta$ -adrenergic receptor ( $\beta$ AR) inotropic reserve. We tested whether prolonged LV unloading of failing rabbit hearts by use of a heterotopic transplantation technique could lead to recovery and whether adenoviral gene transfer of a  $\beta_2$ AR transgene (Adv- $\beta_2$ AR) could alter this process.

**Methods and Results**—Heart failure was induced by coronary artery ligation in adult New Zealand White rabbits. After 4 weeks, failing hearts were heterotopically transplanted into recipient rabbits, allowing normal coronary perfusion but complete LV unloading. We also placed an LV latex balloon for remote access and in vivo physiological analysis. We found that there was reversal of signaling and functional abnormalities after 30 days of unloading. In another set of failing hearts, we randomly delivered, at the time of transplantation, either  $2 \times 10^{11}$  viral particles of Adv- $\beta_2$ AR or saline via the coronary arteries. Sham-operated animals with nonfailing hearts served as controls. After 5 days of unloading, in vivo LV contractility (LV dP/dt<sub>max</sub>) and relaxation (LV dP/dt<sub>min</sub>) were significantly decreased in saline-treated failing hearts compared with control nonfailing hearts ( $P < 0.05$ ). In failing hearts treated with Adv- $\beta_2$ AR, however, LV dP/dt<sub>max</sub> and LV dP/dt<sub>min</sub> were improved in response to higher preloads ( $P < 0.05$ ) and  $\beta$ AR stimulation ( $P < 0.01$ ).

**Conclusions**—Heterotopic transplantation in the rabbit does allow recovery of the failing heart, and  $\beta_2$ AR overexpression acutely enhances this functional improvement. Accordingly, genetic manipulation of  $\beta$ AR signaling may represent a novel molecular adjunct to mechanical assistance to facilitate functional myocardial recovery. (*Circulation*. 2002;106:124-129.)

**Key Words:** heart failure ■ receptors, adrenergic, beta ■ remodeling ■ heart-assist device ■ gene therapy

Alterations that take place in the  $\beta$ -adrenergic receptor ( $\beta$ AR) system during progression of heart failure (HF) are well characterized.<sup>1</sup> Typically,  $\beta_1$ AR density is selectively reduced and the remaining  $\beta_1$ - and  $\beta_2$ ARs are desensitized.<sup>2</sup> Studies in transgenic mice have revealed that cardiac overexpression of  $\beta_2$ ARs can be a mechanism for enhanced inotropic support for the heart.<sup>3,4</sup> These studies have also been extended to normal rabbits in which intracoronary delivery of a  $\beta_2$ AR-containing adenovirus (Adv- $\beta_2$ AR) has improved in vivo LV function,<sup>5,6</sup> suggesting that overexpression of  $\beta_2$ ARs may represent a novel strategy to improve ventricular function in patients with HF. In contrast to the apparent therapeutic effect of  $\beta_2$ ARs,  $\beta_1$ AR overexpression in the hearts of transgenic mice, even at low levels, leads to progressive early cardiomyopathy and HF.<sup>7</sup>

Treatment of patients with end-stage HF involves different modalities, including mechanical support with a left ventricular assist device (LVAD) and cardiac transplantation. Recently, significant improvement in ventricular function has been reported in some patients assisted with a mechanical

pump, and surprisingly, successful weaning from the LVAD was occasionally possible.<sup>8-12</sup> Little is known regarding the mechanisms that might predict this functional recovery,<sup>10</sup> although contractile response to  $\beta$ AR stimulation improved in samples obtained from human ventricles unloaded with an LVAD.<sup>13,14</sup> Therefore, a better understanding of the “reverse remodeling” process, ie, the mechanisms involved in such functional recovery during ventricular unloading, may lead to new molecular approaches that could provide novel hemodynamic support strategies to favor myocardial recovery and potentially successful weaning from LVADs without orthotopic transplantation.

In the present study, we wanted to determine whether heterotopic transplantation of failing rabbit hearts could represent a model of LV unloading and support functional recovery. Moreover, because  $\beta$ AR stimulation is the most powerful means of enhancing ventricular contractility, we investigated the potential benefit of combining genetic augmentation of  $\beta_2$ AR signaling and ventricular unloading for the reverse remodeling process.

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**TABLE 1. Comparison of LV dP/dt<sub>max</sub>, LV dP/dt<sub>min</sub>, and LV Systolic Pressure in Failing (4 Weeks Post-MI) or Control (4 Weeks Post-Sham) Hearts at Day 1 After Transplantation**

	HF (n=10)	Control Hearts (n=7)
LV dP/dt <sub>max</sub> , mm Hg/s		
Baseline	707.5±83.8*	986.8±93.8
+0.3 mL	810.0±99.3†	1342.2±99.2
ISO+0.3 mL	1200.6±134.7†	2075.6±203.4
LV dP/dt <sub>min</sub> , mm Hg/s		
Baseline	-718.6±67.7*	-945.7±102.9
+0.3 mL	-853.2±88.8†	-1302.3±74.4
ISO+0.3 mL	-1208.7±138.3†	-1916.1±151.6
LV systolic pressure, mm Hg		
Baseline	42.8±2.9	50.9±5.6
+0.3 mL	70.0±5.2*	86.3±7.6
ISO+0.3 mL	78.8±7.0*	108.8±10.8

Baseline represents conditions in which the LV balloon and volume was adjusted to an LV end-diastolic pressure of 0 mm Hg; +0.3 mL, a volume of 0.3 mL; and ISO+0.3 mL, both ISO (0.1 μg · kg<sup>-1</sup> · min<sup>-1</sup>) and a 0.3 mL increased preload.

\*P<0.05, †P<0.005 vs control hearts.

**Methods**

**Adenoviral Transgenes**

We used a first-generation E1/E3-deleted replication-deficient serotype 5 adenovirus as previously described.<sup>5,15</sup> The β<sub>2</sub>AR transgene (Adv-β<sub>2</sub>AR) and the marker transgene β-galactosidase (Adv-βGal) were driven by the cytomegalovirus promoter. Virus was thawed and mixed with PBS to a final volume of 2 mL immediately before use.

**Induction of HF**

Adult male New Zealand White rabbits (3 kg) were used, and all procedures were performed in accordance with the regulations adopted by the National Institutes of Health and approved by the Animal Care and Use Committee of Duke University. A myocardial infarction (MI) was induced by a ligation of the first marginal branch of the left circumflex coronary artery as previously described.<sup>16,17</sup> The control group included sham-operated animals in which only a thoracotomy and pericardiectomy were performed.

**Heterotopic Transplantation and Gene Delivery**

Four weeks after left circumflex coronary artery ligation, donor animals were anesthetized with ketamine (60 mg/kg) and acepromazine (1.0 mg/kg) and ventilated. The hearts were exposed via a clam-shell thoracic incision. HF was confirmed by global dilation of the heart, the presence of pleural effusion and/or ascites, and the presence of a large infarcted area estimated to be ≥30% of the LV free wall. The donor hearts were then arrested by intracoronary perfusion with 30 mL of University of Wisconsin cold cardioplegic solution and quickly harvested and maintained at 4°C as described.<sup>18,19</sup> Explanted hearts were randomized to receive either adenovirus or PBS. The hearts were then transplanted into the neck of recipient rabbits as described.<sup>18</sup> Total ischemic time was ≈45 minutes. The hearts resumed vigorous contraction within 3 minutes of reperfusion. Dexamethasone (4 mg/kg) was administered intravenously before reperfusion and then intramuscularly on a daily basis. In a subgroup, an LV biopsy was taken from the donor heart for biochemical comparison with paired samples obtained 5 days after unloading.

**LV Functional Assessment**

In another subgroup of experiments, we positioned an LV latex balloon into the left atrium connected to tubing conducted under the skin to the subscapular region. To measure cardiac function, animals

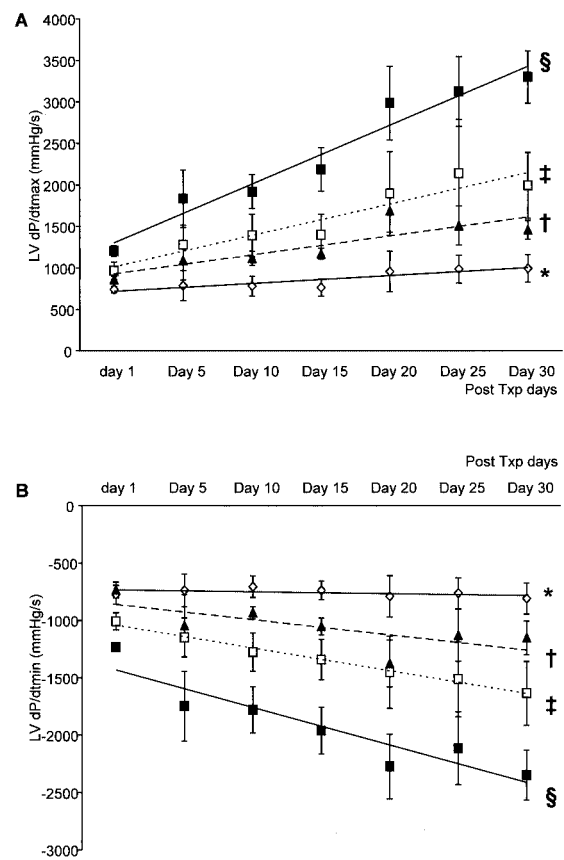
were lightly anesthetized, and the extremity of the tubing was retrieved and connected to a Y-connector that allowed adjustment of the LV end-diastolic volume from one port and the introduction of a high-fidelity pressure transducer (Millar Instruments) through the other port. Baseline LV end-diastolic volume was normalized to a balloon volume providing an end-diastolic pressure of 0 mm Hg. LV function was assessed at days 1, 3, and 5 under 2 different preload conditions (baseline and +0.3 mL). Response to βAR stimulation was assessed by an intravenous infusion of 0.1 μg · kg<sup>-1</sup> · min<sup>-1</sup> isoproterenol (ISO). At day 5, animals were euthanized, and infarction size was calculated as a percentage of the entire free wall as described.<sup>17</sup> In a separate group of animals, longer-term effects of unloading were also examined by measurement of LV function every 5 days until day 30.

**Determination of Myocardial βAR Density**

Cardiac sarcolemmal membranes were prepared and total βAR density was determined as previously described.<sup>3,5</sup>

**Statistical Analysis**

Data are expressed as mean±SEM. Paired Student's *t* tests were used for comparison of βAR density before and after transplantation.



**Figure 1.** Progression of LV recovery over a 30-day period of unloading of failing hearts (4 weeks after MI, n=5). A, LV contractility (LV dP/dt<sub>max</sub>). B, LV relaxation (LV dP/dt<sub>min</sub>). Basal conditions were defined as the LV end-diastolic volume necessary to generate an LV end-diastolic pressure of 0 mm Hg. Basal function (◇) did not improve over the 30-day unloading period (\*P=NS; 1-way ANOVA for repeated measures). Response to a perfusion of 0.1 μg · kg<sup>-1</sup> · min<sup>-1</sup> ISO (▲) improved slightly over time (†P<0.05 for LV dP/dt<sub>max</sub>, P=NS for LV dP/dt<sub>min</sub>). The function in loaded LV (basal+0.3 mL end-diastolic volume) improved significantly over time (□; ‡P<0.05 for LV dP/dt<sub>max</sub>, P<0.01 for LV dP/dt<sub>min</sub>). ISO stimulation (0.1 μg · kg<sup>-1</sup> · min<sup>-1</sup>) of loaded LV (basal+0.3 mL end-diastolic volume) improved to the greatest extent over the 30-day period of LV unloading (■; §P<0.001 for LV dP/dt<sub>max</sub> and for LV dP/dt<sub>min</sub>). Txp indicates transplantation.

One-way and 2-way ANOVAs were used to compare LV function in transplanted hearts. For all tests, a value of  $P < 0.05$  was considered significant.

## Results

### Assessment of HF

A total of 42 rabbit hearts were successfully transplanted into the neck of recipient animals and studied regularly until euthanization at day 5 or day 30. Hearts with an infarct size estimated to be  $< 30\%$  of the LV free wall (at the time of death) and that were not accompanied by ascites and/or pleural effusion were not considered for transplantation. Importantly, no significant difference in infarct size was observed between MI hearts that received Adv- $\beta_2$ AR ( $35.0 \pm 2.3\%$  of the LV free wall,  $n=4$ ) and MI hearts treated with PBS ( $38.9 \pm 1.9\%$ ;  $n=6$ ,  $P=NS$ ).

In vivo LV hemodynamics was measured in 4-week post-MI hearts via the inserted latex balloon at day 1 after transplantation. This function 24 hours after transplant allowed the effect of cardioplegic arrest and surgery to become less important.<sup>19,20</sup> All indices of LV function were significantly decreased compared with the function of sham-operated transplanted hearts ( $n=7$ ), confirming that these hearts were in HF (Table 1).

### Functional Recovery of Unloaded (Transplanted) Failing Hearts

We tested whether chronic unloading via heterotopic transplantation could lead to reversal of LV dysfunction. To aid against rejection,<sup>18</sup> we treated rabbits with a daily dose of dexamethasone, and LV function was assessed every 5 days until day 30 after surgery. Basal LV function did not change over the 30-day period of unloading in hearts transplanted 4 weeks after MI. In post-MI hearts, however, both LV contractility (LV  $dP/dt_{max}$ ) and relaxation (LV  $dP/dt_{min}$ ) progressively increased after ISO stimulation during the 4 weeks of unloading (Figure 1). Moreover, the response to an increased preload also improved in these failing hearts during the 30 days of unloading, and interestingly, the combination of both

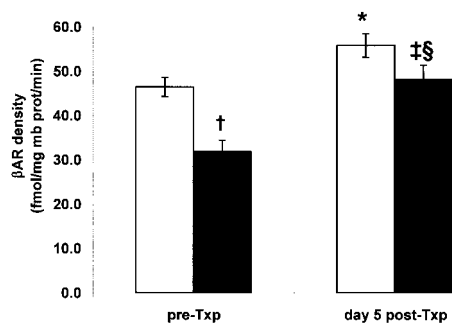
increased preload and ISO provided the highest improvement over the 30 days of ventricular unloading (Figure 1).

### Restoration of $\beta$ AR Density in Unloaded Failing Ventricles

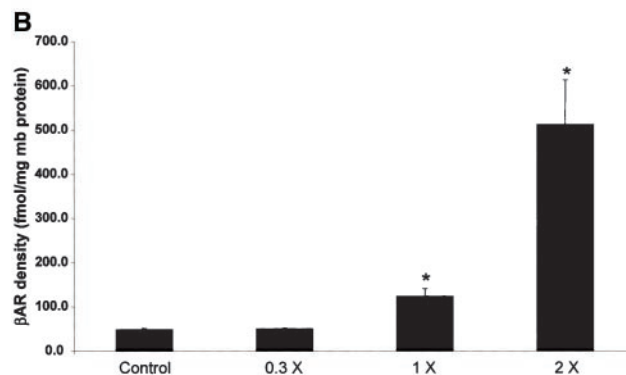
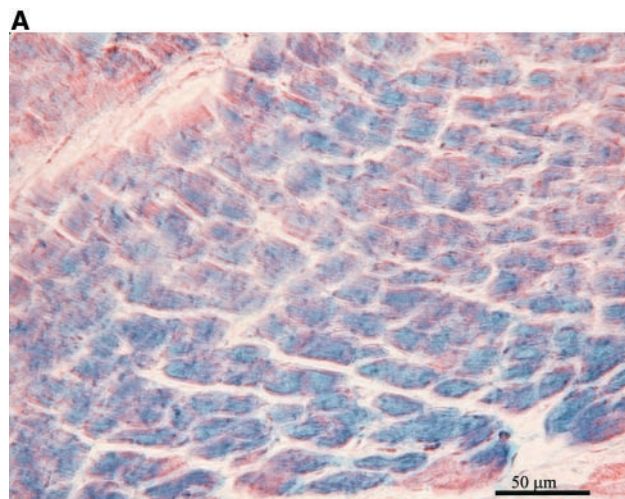
As in other models of HF,  $\beta$ AR density in LV biopsies obtained at the time of transplantation of the failing hearts (post-MI) was decreased ( $31.8 \pm 2.6$  fmol/mg protein,  $n=6$ ) compared with control (sham) ( $46.4 \pm 2.2$  fmol/mg protein,  $n=4$ ,  $P < 0.005$ ) (Figure 2). Interestingly,  $\beta$ AR density was restored to normal levels ( $48.1 \pm 3.3$  fmol/mg protein,  $n=6$ ,  $P < 0.0005$  versus pretransplantation) in membranes purified from the same failing hearts after 5 days of unloading via heterotopic transplantation (Figure 2).

### Transgene Expression

Five days after ex vivo delivery of  $2 \times 10^{11}$  total viral particles (TVP) of Adv- $\beta$ Gal, the transgene was robustly expressed in the transplanted heart, as confirmed by histological galactosidase staining (Figure 3A). Expression of Adv- $\beta_2$ AR was dose-dependent, and both  $1 \times 10^{11}$  (1X) and  $2 \times 10^{11}$  (2X) TVP significantly increased  $\beta$ AR density, with the 2X dose



**Figure 2.** Comparison of  $\beta$ AR density in membrane (mb) preparations obtained from LV before heterotopic transplantation (pre-Txp) and 5 days after LV unloading (post-Txp) in normal (□;  $n=4$ ) and failing (■,  $n=6$ ) hearts. In normal hearts,  $\beta$ AR density increased from  $46.4 \pm 2.2$  to  $55.8 \pm 2.7$  fmol/mg mb protein ( $*P < 0.05$ ).  $\beta$ AR density was decreased significantly in failing hearts before transplantation ( $31.8 \pm 2.6$  fmol/mg mb protein;  $\dagger P = 0.002$ ) but increased to  $48.1 \pm 3.3$  fmol/mg mb protein after 5 days of unloading ( $\ddagger P < 0.0001$ ), which was similar to normal unloaded hearts ( $\$P = NS$ ).



**Figure 3.** Myocardial transgene expression 5 days after cardiac heterotopic transplantation (Txp) and intracoronary gene delivery. A,  $\beta$ -Gal staining in a representative normal LV 5 days after Txp and delivery of  $2 \times 10^{11}$  TVP of Adv- $\beta$ Gal. B,  $\beta$ AR density in membrane (mb) preparation obtained from Txp failing hearts and comparison between 3 doses of Adv- $\beta_2$ AR:  $0.3 \times 10^{11}$  TVP ( $n=6$ ),  $1 \times 10^{11}$  TVP ( $n=3$ ), and  $2 \times 10^{11}$  TVP ( $n=4$ ). Control hearts received PBS only ( $n=6$ ).  $*P < 0.001$  vs control.

**TABLE 2. Comparison of LV dP/dt<sub>max</sub>, LV dP/dt<sub>min</sub>, and LV Systolic Pressure 5 Days After LV Unloading Between Failing Hearts (4 Weeks Post-MI) That Received Either PBS (HF+PBS) or Adv-β<sub>2</sub>AR (HF+βAR) at the Time of Transplantation, and Control Hearts (4 Weeks Post-Sham) That Received PBS (Control Hearts+PBS)**

	HF+PBS (n=6)		HF+βAR (n=4)		Control Hearts+PBS (n=7)	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
<b>LV dP/dt<sub>max</sub></b>						
Baseline	678.5±97.2	849.3±120.8	909.5±170.0	1349.1±377.8	986.8±93.8	1527.4±142.4†
+0.3 mL	806.6±122.7	935.0±139.0	1021.3±218.2	2224.8±449.0*	1342.2±99.2	2289.8±135.3‡
ISO+0.3 mL	1253.5±169.6	1350.1±175.5	1548.2±409.5	2652.6±393.9*	2075.6±203.4	3230.5±222.8‡
<b>LV dP/dt<sub>min</sub></b>						
Baseline	-652.3±74.4	-764.3±93.5	-942.8±109.9	-1157.0±233.7	-945.7±102.9	-1218.1±95.7†
+0.3 mL	-834.1±104.8	-892.3±126.9	-1026.1±170.4	-1623.7±271.0*	-1302.3±74.4	-1879.0±70.7‡
ISO+0.3 mL	-1257.4±180.5	-1203.7±153.2	-1459.6±362.2	-1965.5±229.2*	-1916.1±151.6	-2457.4±115.9‡
<b>LV systolic pressure</b>						
Baseline	41.5±3.8	45.7±3.6	53.1±7.3	68.4±15.1	50.9±5.6	76.1±6.8†
+0.3 mL	65.4±6.8	68.9±6.5	85.5±7.9	139.0±14.8†	86.3±7.6	149.5±9.6‡
ISO+0.3 mL	75.7±10.6	77.7±10.4	103.5±17.1	158.1±16.7†	108.8±10.8	163.7±8.5‡

Conditions and units are identical to Table 1.

\* $P<0.05$ , † $P<0.01$ , ‡ $P<0.001$  vs group of HF+PBS.

increasing it to  $511.6\pm 101.5$  fmol/mg membrane protein (n=4). This represents >8 times the value measured in control hearts (Figure 3B;  $P<0.005$ ). Accordingly, we used  $2\times 10^{11}$  TVP as our unloaded heart dose.

### β<sub>2</sub>AR Gene Transfer Improves Functional Recovery of Unloaded Failing Hearts

Although baseline function did not differ significantly between failing hearts that received the β<sub>2</sub>AR transgene (n=4) and those that received PBS (n=6), we found an improved response to mechanical stimulation when we increased preload to 0.3 mL above baseline volume ( $P<0.05$ , Table 2 and Figure 4). We also observed a significant functional improvement in response to βAR stimulation after 5 days of LV unloading ( $P<0.05$ , Table 2 and Figure 4). Moreover, maximal LV pressure generated during systole was significantly increased in unloaded failing hearts previously treated with Adv-β<sub>2</sub>AR compared with those that received PBS ( $P<0.005$ , Figure 5). In fact, functional response to ISO stimulation was virtually identical to that obtained from nonfailing (sham, n=7) unloaded hearts (Figure 5B).

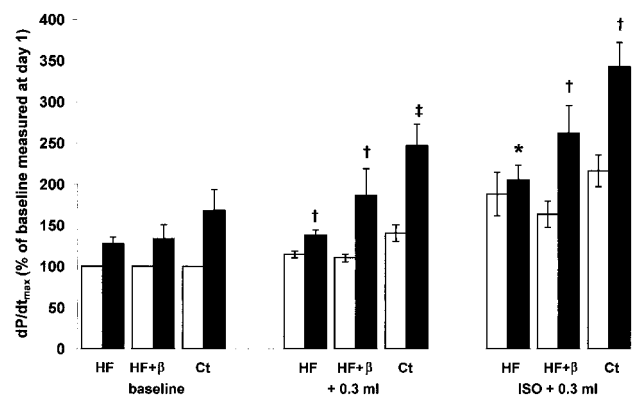
### Restoration of βAR Density Is Independent of Cardiac Denervation

Because the transplantation technique renders the grafted heart denervated, restoration of βAR density might be a result of the absence of direct neural stimulation rather than unloading. To test this, we compared the function of normal transplanted hearts in which the LV balloon was kept inflated to maintain LV loading with that of unloaded transplanted normal hearts in which the balloon was deflated, except for data recordings at day 1 and day 5. Although no baseline difference was observed between loaded and unloaded hearts after 5 days, both βAR and preload-induced function progressively improved in unloaded hearts, whereas no change occurred in loaded hearts (Figure 6A). In addition, βAR

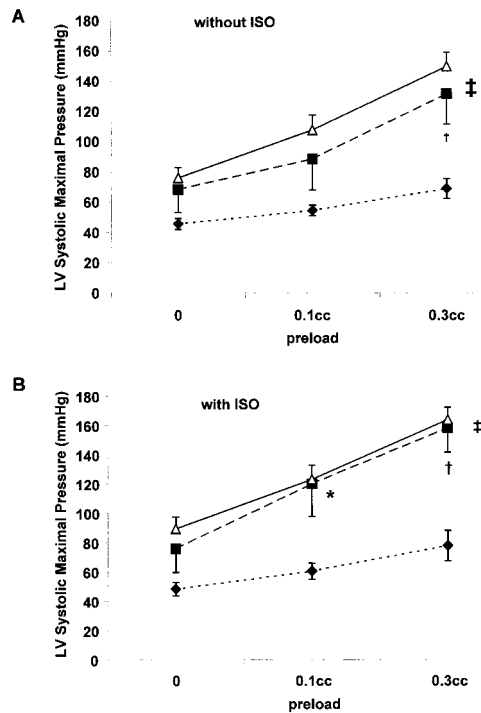
density significantly increased in unloaded hearts compared with nontransplanted hearts, whereas no difference was observed in transplanted hearts in which the LV balloon was kept inflated (Figure 6B). Thus, restoration of βAR density as well as functional improvement appears to be due to mechanical unloading rather than denervation.

## Discussion

In the present study, we used the heterotopic transplantation model to simulate ventricular unloading. Using this model, we were able to reproduce the functional improvements observed in human failing hearts unloaded by use of an LVAD. Moreover, we demonstrate the benefit of gene-



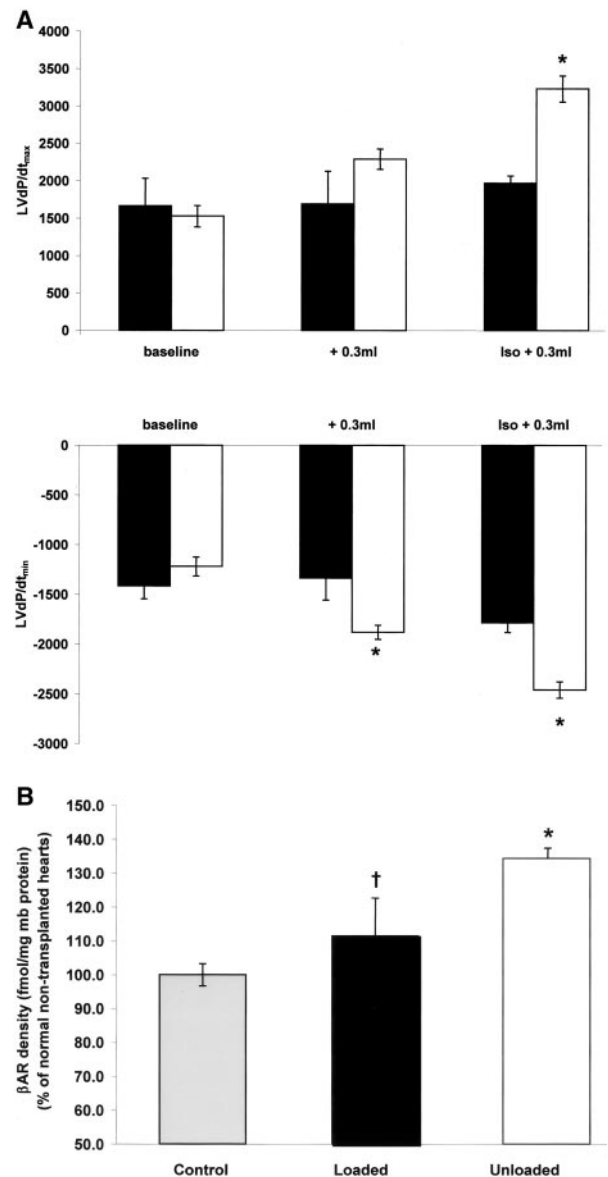
**Figure 4.** Comparison of contractility between day 1 (□) and day 5 (■) after heterotopic transplantation of normal hearts (Ct; n=7), failing hearts treated by transplantation and treated with Adv-β<sub>2</sub>AR (HF+β; n=4), and failing transplanted hearts that received PBS (HF; n=6). Shown is contractility at baseline (LV end-diastolic pressure=0 mm Hg), to increased preload conditions (+0.3 mL), and after βAR stimulation with 0.1 μg · kg<sup>-1</sup> · min<sup>-1</sup> ISO, and under a combination (ISO+0.3 mL). Results are expressed as percentage of the basal value measured at day 1 after transplantation. \* $P=NS$ , † $P<0.05$ , ‡ $P<0.01$  (day 5 vs day 1).



**Figure 5.** LV systolic maximal pressure (5 days after transplantation) in response to increased preload conditions and (A) without or (B) with ISO stimulation ( $0.1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in normal hearts ( $\Delta$ ;  $n=7$ ) and failing hearts that received Adv- $\beta_2$ AR ( $\blacksquare$ ;  $n=4$ ) or PBS ( $\blacklozenge$ ;  $n=6$ ). \* $P<0.05$ , † $P<0.005$  (Adv- $\beta_2$ AR vs PBS). ‡ $P=NS$  (ANOVA, Adv- $\beta_2$ AR transplanted hearts vs normal transplanted hearts).

mediated modulation of  $\beta_2$ ARs to restore functional response to  $\beta$ AR stimulation as well as to increased preload. Thus, our data indicate that it may be advantageous to combine mechanical unloading with a molecular therapeutic modality to hasten and enhance myocardial functional recovery. As described previously, not all patients may recover sufficient ventricular strength to allow weaning from their LVAD<sup>8–12</sup> and prolonged recovery of their myocardial contractile function.<sup>21</sup> In the present study, failing hearts required 1 month of unloading after a large LV infarction before we were able to measure functional improvement. In contrast, failing hearts that received intracoronary Adv- $\beta_2$ AR demonstrated significant LV functional improvement within the first week of ventricular unloading. Thus, Adv- $\beta_2$ AR gene therapy to the unloaded failing heart may represent a novel form of “molecular assistance” to provide beneficial synergy to mechanical assistance.

Previous reports have suggested a role for  $\beta$ AR signaling in the reverse remodeling process that occurs in mechanically assisted failing hearts.<sup>13,14</sup> For example, response to  $\beta$ AR stimulation was increased in human myocytes isolated from hearts unloaded with an LVAD.<sup>13,14</sup> In the present study,  $\beta$ AR density was increased in normal hearts 5 days after heterotopic transplantation, and we verified that these changes were independent of denervation. Five days was not sufficient, however, to permit any significant functional improvement due to unloading. Importantly, overexpression of  $\beta_2$ ARs to  $\approx 8$ -fold over normal via adenovirus-mediated



**Figure 6.** Contractility and  $\beta$ AR density in chronically loaded and unloaded control transplanted hearts. A, LV  $dP/dt_{\text{max}}$  and LV  $dP/dt_{\text{min}}$  of normal hearts 5 days after transplantation. Comparison is made between unloaded ( $\square$ ;  $n=5$ ) and loaded ( $\blacksquare$ ;  $n=3$ ) hearts as described in the text. B,  $\beta$ AR density in these loaded ( $n=3$ ) vs unloaded ( $n=3$ ) transplanted hearts. Values are expressed as percentage of a control group of normal nontransplanted hearts ( $n=3$ ). \* $P<0.005$  (unloaded hearts vs control nontransplanted hearts). † $P=NS$  (loaded hearts vs control nontransplanted hearts).

$\beta_2$ AR gene delivery at the time of transplantation was accompanied by significant increases in both contractility and relaxation in response to preload and  $\beta$ AR agonist stimulation within just 5 days. Thus, manipulation of  $\beta_2$ AR signaling appears to be able to play a critical role in the reverse remodeling process by triggering functional recovery of unloaded failing hearts.

It is noteworthy that in transgenic mice, cardiac overexpression of  $\beta_2$ ARs at extraordinary high levels ( $>150$ -fold) was associated with cardiomyopathy, whereas cardiac  $\beta_2$ AR overexpression to up to  $\approx 100$ -fold showed no pathology.<sup>3,4</sup> These studies in transgenic

mice led to the hypothesis that  $\beta_2$ AR overexpression would be beneficial in HF. Indeed,  $\beta_2$ AR gene delivery to normal rabbit hearts also enhanced function, although  $\beta_2$ AR overexpression was much lower.<sup>5,6</sup> Moreover,  $\beta_2$ AR overexpression, at levels seen in the present study, in cultured failing rabbit cardiomyocytes did improve abnormal  $\beta$ AR signaling; however, functional indices were not measured. Importantly, actual treatment of a failing heart with  $\beta_2$ AR gene transfer, such as in the present study, has not previously been performed. In this study as well as in our previous adenovirus-mediated gene delivery studies, we have not seen cardiac  $\beta_2$ AR overexpression exceed  $\approx 15$ -fold.<sup>5,6</sup> Thus, we do not believe that this methodology would lead to "toxic" levels of  $\beta_2$ AR overexpression, but importantly, we appear to be able to achieve "therapeutic" levels of  $\beta_2$ ARs in the heart.

It is important to note the appreciation that signaling through  $\beta_1$ ARs and  $\beta_2$ ARs are qualitatively and quantitatively different, which was reviewed recently.<sup>22</sup> In contrast to the beneficial cardiac effects after  $\beta_2$ AR overexpression (up to 100-fold), transgenic overexpression of  $\beta_1$ ARs in the mouse heart even at low levels (15- to 30-fold) produces significant pathology, including cardiomyopathy and HF. Moreover, some data indicate that these 2  $\beta$ AR subtypes can exhibit opposing effects in the heart, including the induction of apoptosis.<sup>22</sup> Thus, as this study indicates, selective enhancement of  $\beta_2$ AR signaling may be therapeutic in the failing heart, because we have shown that  $\beta_2$ AR overexpression can hasten the functional recovery of unloaded failing hearts.

This study does present some limitations. We did not specifically address the arrhythmic activity in the transplanted hearts that may be expected after  $\beta_2$ AR overexpression. Another concern in the interpretation of these results is immune rejection, although no significant signs of immune rejection were detected after 5 days of transplantation. Inflammatory reactions might trigger adverse reactions that could also influence the interactions between reverse remodeling, adenovirus-mediated modification of genetic expression, and subsequent functional changes. In addition, the nature of adenoviruses is such that they allow gene expression for a limited period of time and therefore may not be appropriate for long-term studies of reverse remodeling. This could be an advantage, however, because permanent overexpression may be deleterious after adequate functional recovery is achieved. Finally, in this model of unloading, the failing heart is removed from its environment and placed into a healthy recipient. Although we demonstrated that functional and  $\beta$ AR density recoveries were independent of cardiac denervation, other humoral molecules present in the HF patient may influence ventricular remodeling.

Nevertheless, our study clearly demonstrates the potential usefulness of  $\beta_2$ AR augmentation to aid in the reverse remodeling process that occurs in unloaded hearts, because this may represent a beneficial molecular adjunct to mechanical unloading to improve functional recovery of severely dysfunctional hearts. Moreover, our study demonstrates that heterotopic transplantation can reasonably assimilate mechanical unloading and may thus represent a suitable model to study the functional and biochemical effects of adjunct therapeutic modalities.

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