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Intracoronary Adenovirus-Mediated Delivery and Overexpression of the β_2 -Adrenergic Receptor in the Heart Prospects for Molecular Ventricular Assistance

Ashish S. Shah, MD; R. Eric Lilly, MD; Alan P. Kypson, MD; Oliver Tai, BS; Jonathan A. Hata, BA; Anne Phippen, BS; Scott C. Silvestry, MD; Robert J. Lefkowitz, MD; Donald D. Glower, MD; Walter J. Koch, PhD

Background—Genetic modulation of ventricular function may offer a novel therapeutic strategy for patients with congestive heart failure. Myocardial overexpression of β_2 -adrenergic receptors (β_2 ARs) has been shown to enhance contractility in transgenic mice and reverse signaling abnormalities found in failing cardiomyocytes in culture. In this study, we sought to determine the feasibility and in vivo consequences of delivering an adenovirus containing the human β_2 AR cDNA to ventricular myocardium via catheter-mediated subselective intracoronary delivery.

Methods and Results—Rabbits underwent percutaneous subselective catheterization of either the left or right coronary artery and infusion of adenoviral vectors containing either a marker transgene (Adeno- β Gal) or the β_2 AR (Adeno- β_2 AR). Ventricular function was assessed before catheterization and 3 to 6 days after gene delivery. Both left circumflex- and right coronary artery-mediated delivery of Adeno- β_2 AR resulted in ≈ 10 -fold overexpression in a chamber-specific manner. Delivery of Adeno- β Gal did not alter in vivo left ventricular (LV) systolic function, whereas overexpression of β_2 ARs in the LV improved global LV contractility, as measured by dP/dt_{\max} , at baseline and in response to isoproterenol at both 3 and 6 days after gene delivery.

Conclusions—Percutaneous adenovirus-mediated intracoronary delivery of a potentially therapeutic transgene is feasible, and acute global LV function can be enhanced by LV-specific overexpression of the β_2 AR. Thus, genetic modulation to enhance the function of the heart may represent a novel therapeutic strategy for congestive heart failure and can be viewed as molecular ventricular assistance. (*Circulation*. 2000;101:408-414.)

Key Words: gene therapy ■ myocardium ■ receptors, adrenergic, β ■ ventricles ■ heart failure ■ signal transduction

Novel treatment strategies for ventricular dysfunction are of great importance, and the area of gene therapy is gaining increasing attention. Traditional approaches to the treatment and palliation of ventricular dysfunction and failure include medical management,¹ myocardial revascularization,² valve repair,³ and mechanical assist and transplantation.⁴ Although there have been advances in these therapies, congestive heart failure (CHF) remains a leading cause of death in the United States and worldwide.⁵ Thus, a molecular approach for treating the failing heart is attractive. Early work in cardiac gene therapy has concentrated on 2 distinct problems: reliable methods of delivery to working myocardium and identification of potential molecular targets. First, work by several groups demonstrated the feasibility of delivering transgenes via direct intramyocardial injection,^{6,7} ex vivo perfusion,^{8,9} and finally a transluminal intracoronary approach.¹⁰ Second, a spectrum of molecular targets has emerged through work done in genetically engineered mice.¹¹

One molecular target identified is the overexpression of the β_2 -adrenergic receptors (β_2 ARs). Transgenic mice with cardiac-specific overexpression of the human β_2 AR at either >100 -fold over endogenous β_2 AR density¹² or significantly lower overexpression¹³ have enhanced contractility without overt pathological conditions. These models were developed to replace receptors that are lost during the development of CHF. In the failing heart, there is a 50% reduction of myocardial β -adrenergic receptors (β -ARs), with remaining receptors being functionally uncoupled.¹⁴ In addition to the positive phenotype of the β_2 AR-overexpressing mice, adenovirus-mediated overexpression of β_2 ARs in failing rabbit ventricular cardiomyocytes in culture has resulted in the functional rescue of the signaling abnormalities present in failing heart cells.¹⁵ Thus, genetically replacing lost β -ARs in the failing heart represents a potentially novel therapeutic strategy to increase inotropy.

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The primary hurdle to testing the feasibility of β_2 AR gene transfer in vivo is the development of a clinically relevant gene delivery system. Recent work by our laboratory¹⁶ and others¹⁷ has shown that it is possible to deliver transgenes globally to the myocardium by adenoviral vectors. In these 2 studies, adenoviruses were delivered via a surgically invasive approach in which the transgenes are injected into the left ventricular (LV) cavity while the aorta is cross-clamped, directing the adenoviral solution to perfuse the coronary arteries.^{16,17} Using this method, we showed that overexpression of β_2 ARs in the rabbit heart does enhance global in vivo LV function.¹⁶ Because this method of gene delivery has its limitations, we explored the feasibility of delivering the β_2 AR transgene to the rabbit heart in vivo via percutaneous subselective coronary catheterization and injection. A previous report showed that transluminal intracoronary artery delivery of marker transgenes in the rabbit is possible; however, that report did not include a study of myocardial function.¹⁰ The purpose of our study was to develop a reproducible percutaneous subselective intracoronary artery delivery method for efficient ventricle-targeted in vivo gene transfer of adenoviral transgenes to rabbit myocardium. Furthermore, we investigated whether ventricular overexpression of the β_2 AR in the rabbit heart could alter biochemical and in vivo cardiac function.

Methods

Adenoviral Constructs

Adenoviral constructs using a "first-generation" E1/E3-deleted replication-deficient adenovirus have been described previously.^{9,15} The β_2 AR construct (Adeno- β_2 AR) and the marker transgene β -galactosidase (Adeno- β Gal) were driven by the CMV promoter. Large-scale preparations of these adenoviruses were purified from infected Epstein-Barr nuclear antigen-transfected 293 cells as described.^{9,15}

Percutaneous Intracoronary Adenovirus Delivery and Cardiac Functional Assessment

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the NIH. Forty-seven adult male New Zealand White rabbits (3 kg) were used in the present study. At the time of the initial study, animals were sedated with ketamine (50 mg/kg) and acepromazine (0.25 mg/kg), and an incision was made over the right neck to expose the right carotid and jugular vessels. A 2.5F micromanometer (Millar Inc) was placed into the LV cavity via the carotid artery under fluoroscopic guidance, and a 22-gauge angiocatheter was placed into the jugular vein. The micromanometer was coupled to a PC-based data acquisition system (Physiological Systems Inc, and LV pressure was obtained at baseline and after the infusion of isoproterenol at $0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 20 minutes as described.¹⁶ All hemodynamic studies were performed in a closed-chest model with an intact pericardium. After ventricular functional assessment of individual rabbits, a 4.5F radial arterial line sheath (Cook Inc) was placed into the carotid artery, and a 3F coronary catheter (Cook Inc) was placed into the left circumflex (LCx, n=37) or right coronary artery (RCA, n=10) under fluoroscopic guidance to deliver the adenoviral transgenes. The coronary catheter used to perform these subselective coronary artery adenovirus injections was a simple right-angle catheter with a single distal port (Cook Inc). Before virus infusion, adenosine (1.5 mg), lidocaine (0.33 mg), and heparin (500 U) were given via the jugular vein, and 5×10^{11} total viral particles (TVP) of a replication-deficient adenovirus in 2.5 mL

of PBS at 37°C was injected as a bolus into the respective coronary artery. In addition to adenovirus, a subset of animals (n=4) received only PBS as a control for the delivery technique. All animals were returned to their cages after they had recovered and were fully alert. In 3 days, cardiac hemodynamics of rabbits that received adenovirus or saline were studied as above. Thus, each rabbit served as its own control for cardiac function. All rabbits received methylprednisolone $5.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ IM for 2 days after adenovirus delivery. After the final assessment, animals were euthanized and their hearts rapidly excised. Transmural samples of the LV free wall, right ventricle (RV), septum, left atrium, lung, brain, and liver were frozen in liquid nitrogen and stored for biochemical analysis.

To examine the effects of heart rate (HR) on maximal rate of pressure rise, rabbits (n=4) underwent transvenous atrial pacing via the right jugular vein, and LV pressure was measured as described above. HR was varied from baseline to 330 bpm.

Data Analysis of In vivo Cardiac Function

Analog data were digitized at 200 Hz and analyzed on a VAX workstation (Digital Equipment Corp) with custom software (Physiological Systems Inc). The maximal rate of pressure rise ($\text{dp}/\text{dt}_{\text{max}}$) was computed from the digital pressure waveform as a running 5-point polyorthogonal transformation. All hemodynamic data were derived from the average of 20 steady-state cardiac cycles.¹⁸

β -AR Density

β -AR binding was performed on myocardial sarcolemmal membrane preparations as we have previously described.^{12,16} Total myocardial β -AR density was determined by incubating 25 μg of sarcolemmal membranes with a saturating concentration of ¹²⁵I-labeled cyanopindolol and 20 $\mu\text{mol/L}$ alprenolol to define nonspecific binding.^{12,16} Assays were performed in triplicate, and β -AR density was normalized to milligrams of membrane protein.

β -Galactosidase Staining

After excision, transverse cross sections of myocardium at the midpapillary level were obtained for histological analysis and stored in 30% sucrose solution before paraffin embedding as described.¹⁶ Paraffin-embedded samples were mounted on a cryostat and sectioned into 5- to 10- μm sections, which were then transferred to a glass slide. β -Gal staining was performed by standard procedures as described.^{9,16}

β_2 AR Immunohistochemistry

Frozen myocardial sections were cut at 10 μm for indirect immunofluorescence studies as we have described.^{9,12,16} Briefly, sections were rinsed in PBS and then in PBS with 0.05% Triton X-100 (Triton-PBS), blocked with serum diluent (10% goat serum in PBS with 0.1% BSA and 0.1% sodium azide), and then rinsed for 15 minutes in Triton-PBS before overnight incubation at 4°C with a primary rabbit anti-human β_2 AR antiserum (1:500 dilution in serum diluent). The sections were then washed, incubated for 1 hour in FITC-conjugated goat anti-rabbit immunoglobulin G (1:50 dilution in serum diluent), rinsed in PBS, mounted with sodium iodide (25 g/L) in 1:1 PBS/glycerol solutions, and photographed.^{9,12}

Statistical Analysis

All data are expressed as the mean \pm SEM. In vivo hemodynamic data were compared by a paired Student's *t* test. Unpaired comparisons were made by use of a 1-way ANOVA. For all analyses, a value of $P < 0.05$ was considered to be statistically significant.

Results

Adenovirus-Mediated Myocardial Overexpression of Transgenes After Intracoronary Delivery

We delivered adenoviral transgenes via either the LCx or RCA in a subselective manner. Transgene expression in the rabbit myocardium was assessed 3 days after delivery to

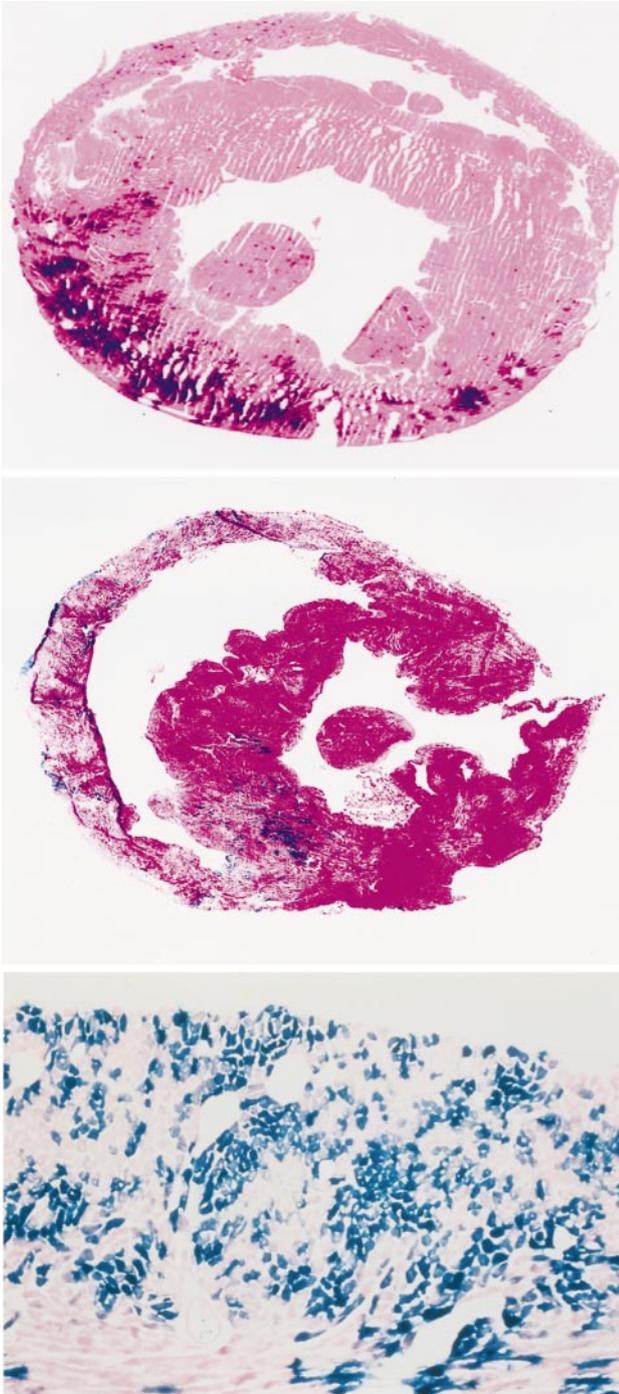


Figure 1. Representative β -Gal staining of (A) LV free wall and (B) RV free wall 3 days after catheterization and delivery of Adeno- β Gal (5×10^{11} TVP) via LCx and RCA, respectively. C, High magnification of LV section demonstrating transgene expression in individual myocytes.

allow time for adequate protein translation and before adenoviral immunological processes were evident. As further protection toward the latter, we treated rabbits (including all control groups) for 2 days with corticosteroids.

To assess the volume of myocardium expressing transgene, we delivered Adeno- β Gal (5×10^{11} TVP) via either the LCx or RCA. Figure 1 shows representative X-Gal-stained cardiac cross sections at low and high power of magnification after

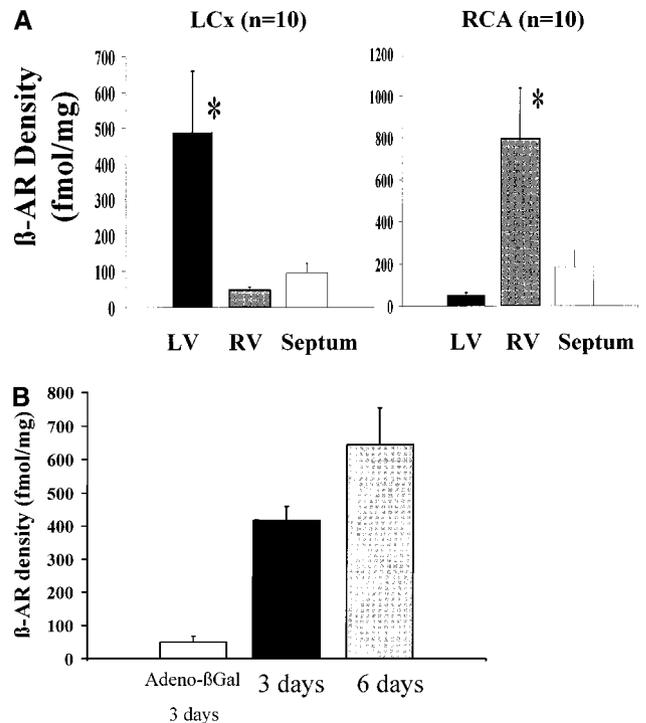


Figure 2. A, β_2 AR overexpression in LV, RV, or septum after delivery of Adeno- β_2 AR (5×10^{11} TVP) via LCx (left, $n=10$) or RCA (right, $n=10$). Chamber-specific β_2 AR overexpression was compared with β -AR density in individual chambers from Adeno- β Gal-treated rabbit hearts (control). * $P < 0.05$ vs density in controls. B, β_2 AR overexpression in LV at 6 days compared with 3-day values.

Adeno- β Gal delivery. Chamber-specific expression of the β Gal gene that corresponded to staining in individual myocytes was evident (Figure 1). Thus, it appears that within the region served by the catheterized coronary artery, complete transmural expression is possible, especially in this adenoviral dose range.

We also studied the delivery and expression of the human β_2 AR, which represents a potentially therapeutic transgene. As with Adeno- β Gal, we delivered 5×10^{11} TVP Adeno- β_2 AR via either the LCx or RCA and initially assessed β -AR density. As shown in Figure 2A, Adeno- β_2 AR delivery in the LCx resulted in significant LV-specific overexpression, whereas catheterization and injection via the RCA results in RV-specific expression. β_2 AR overexpression ranged from ≈ 9 - to 15-fold over endogenous myocardial β -AR density. Expression of the β_2 AR transgene remained elevated at 6 days ($n=4$) after gene delivery (Figure 2B). Importantly, β_2 AR overexpression after in vivo coronary delivery was found to be localized to the sarcolemmal membranes of individual ventricular myocytes as visualized by immunohistochemical staining with an antibody specific for the human β_2 AR (Figure 3). As with the β Gal transgene distribution, β_2 AR overexpression found by immunohistochemistry was diffuse throughout the entire LV after LCx injection, and a representative section of an Adeno- β_2 AR-treated LV is shown in Figure 3.

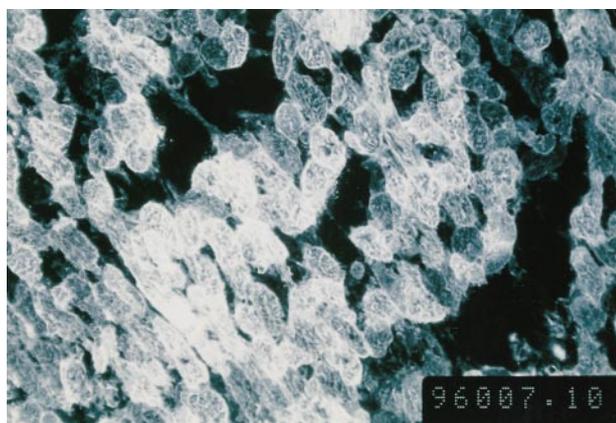


Figure 3. Representative immunohistochemical detection of expressed human β_2 ARs in rabbit LV 3 days after Adeno- β_2 AR (5×10^{11} TVP) delivery via the LCx.

Functional Consequences of Subselective Intracoronary Adenovirus Delivery and Myocardial Overexpression of Adeno- β Gal and Adeno- β_2 AR Transgenes

The hemodynamic consequences of subselective intracoronary delivery of adenoviral transgenes were examined by use of dynamic intracavitary pressure measurements. The maximal first derivative of the LV intracavitary pressure (dP/dt_{\max}) was used as a measure of global LV contractile performance. As described in the Methods, the hemodynamics of each rabbit in the study was measured before and then 3 to 6 days after gene delivery. Thus, each animal served as its own control, increasing the power of the analysis. Furthermore, animals that underwent catheterization and injection of saline served as controls for the delivery technique itself. β -AR density determined for all animals in the study confirmed β_2 AR overexpression. Animals that received Adeno- β Gal via the LCx did not show a significant change in systolic function compared with both precatheterization values and animals injected with saline (Figure 4A). In animals that received Adeno- β_2 AR and subsequently showed β_2 AR overexpression in the LV, a significant increase in baseline dP/dt_{\max} was seen compared with precatheterization values and Adeno- β Gal-treated animals (Figure 4B). LV systolic functional responses to isoproterenol were also significantly greater in β_2 AR-overexpressing animals compared with controls (Figure 4C).

Interestingly, in animals that received either Adeno- β_2 AR or Adeno- β Gal (5×10^{11} TVP each), HRs and LV end-diastolic pressure (EDP) were significantly increased 3 days after gene delivery, whereas there was no change, with either treatment, in systolic blood pressure (Table 1). LV dP/dt_{\min} in Adeno- β Gal-treated rabbits was significantly reduced, whereas no reduction was evident in Adeno- β_2 AR-treated rabbits (Table 1). Hemodynamic values were not altered 3 days after saline delivery including dP/dt_{\max} (Figure 4A), dP/dt_{\min} (before, -2387 ± 148 mm Hg/s versus after, -2152 ± 196 mm Hg/s, $P=NS$), and LV EDP (before, 0.7 ± 0.15 mm Hg versus after, 0.9 ± 0.4 mm Hg, $P=NS$).

LV dP/dt_{\max} measurements can be confounded by changes in HR and afterload. Accordingly, a subset of animals was studied to determine the effect of HR on LV dP/dt_{\max} by use

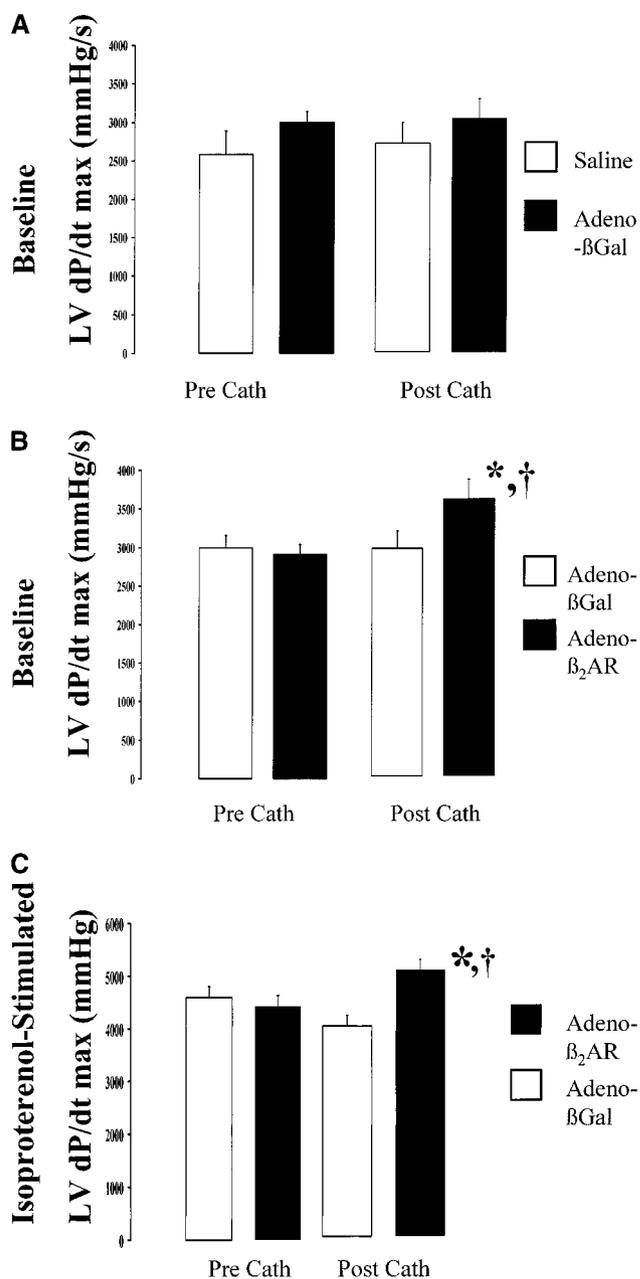


Figure 4. A, Comparison of systolic function (LV dP/dt_{\max}) 3 days after administration and overexpression of Adeno- β Gal (5×10^{11} TVP) via LCx. Post-gene delivery values (Post Cath) are compared with Pre Cath values or animals that received saline (PBS). B and C, Effect of β_2 AR gene delivery on LV systolic performance: B, LV dP/dt_{\max} 3 days after delivery (Post Cath) of Adeno- β_2 AR (5×10^{11} TVP, $n=10$) via LCx vs Pre Cath values ($P=0.007$) and vs rabbits that received 5×10^{11} TVP Adeno- β Gal ($n=10$, $P=0.01$). C, LV contractile response to infusion of isoproterenol ($0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in these rabbits. * $P < 0.05$ vs Pre Cath values; † $P < 0.05$ vs Post Cath values from Adeno- β Gal-treated rabbits.

of transvenous atrial pacing. As shown in Figure 5, the HR effect in the range of 200 to 300 bpm did not significantly increase LV dP/dt_{\max} in a normal rabbit, demonstrating that increased HR is not accountable for the significant increase in basal and isoproterenol-stimulated LV dP/dt_{\max} found in Adeno- β_2 AR-treated rabbits.

TABLE 1. Summary of Hemodynamic Data Before (Pre-Cath) and 3 Days After (Post-Cath) Intracoronary LCx Delivery of Adeno- β_2 AR (n=10) or Adeno- β Gal (n=10) (5×10^{11} TVP)

	Pre-Cath		Post-Cath	
	Adeno- β Gal	Adeno- β_2 AR	Adeno- β Gal	Adeno- β_2 AR
LV dP/dt _{max} , mm Hg/s	2998.8±163	2911.5±188	2837.2±213	3454.8±237*
LV dP/dt _{min} , mm Hg/s	-2641.8±162	-2687.4±120	-2251.3±174*	-2429.6±178
HR, bpm	245±8.3	244±6.2	275±13*	278±8*
LV EDP, mm Hg	0.7±0.28	0.25±0.39	2.6±0.7*	5.8±1.4*
LV SBP, mm Hg	67.1±2.5	69.5±1.8	64±2.5	72.4±2.7

SBP indicates systolic blood pressure.

* $P < 0.05$ vs Pre-Cath.

The functional benefit of β_2 AR overexpression in the LV was also studied 6 days (n=4) after gene delivery. Importantly, basal and isoproterenol-stimulated LV systolic performance remained elevated at 6 days compared with pre-gene delivery hemodynamic measurements (Table 2).

Finally, the effect of β_2 AR overexpression in the RV after Adeno- β_2 AR delivery via the RCA on LV function was also studied. No improvement in LV systolic performance was seen in animals that overexpressed the β_2 AR in the RV (n=6) from precatheterization baseline values (before catheterization, 2725.8 ± 211 versus after catheterization, 2858.7 ± 48 mm Hg/s, $P = \text{NS}$).

Discussion

The present study reports the novel findings that in vivo molecular manipulation of ventricular function by a percutaneous intracoronary gene delivery method is feasible. Accordingly, we have demonstrated that chamber-specific delivery of an adenovirus containing the human β_2 AR via subselective coronary catheterization and injection produces ventricular overexpression of the β_2 AR at a level that increases contractile function in vivo, demonstrating a therapeutic strategy to effectively produce molecular ventricular assistance in CHF.

The β -AR signaling system is an appealing target for cardiac gene therapy, because specific molecular abnormali-

ties have been well described in human CHF and in multiple animal models of failure and cardiac disease.^{11,19} A down-regulation of β -ARs, uncoupling from second messenger systems, and elevation of desensitizing G-protein receptor kinase activity are the fundamental alterations seen in heart failure.^{14-16,19} In myocytes isolated from hearts in CHF, these abnormalities can be reversed by β_2 AR overexpression or G-protein-coupled receptor kinase inhibition by use of transgenes delivered via adenoviral vectors.¹⁵ These studies, as well as studies in transgenic mice, form the basis of our β_2 AR gene therapy strategy.^{11,12,15}

The overexpression of β_2 ARs in the LV free wall appears to improve global LV systolic performance. dP/dt_{max} both at baseline and after isoproterenol administration was increased relative to the precatheterization values in each of the individual rabbits. This is consistent with our findings in a model of biventricular overexpression of β_2 ARs.¹⁶ It is extremely encouraging that the $\approx 20\%$ improvement seen in this study occurred after only LV free wall transfection and a relatively modest 10-fold increase in LV β -AR density. Furthermore, this functional benefit was sustained at 6 days after gene delivery. Previous reports in transgenic mice demonstrated greater improvement in LV contractile function with β_2 AR overexpression. However, these mice had considerably higher levels of β_2 AR overexpression, which was global in nature and not limited to a single chamber, as is the case in the rabbits of this study. This is the first animal model to examine the in vivo effects of chamber-specific overexpression of the β_2 AR in the intact circulation, where ventricular interactions limit the maximal improvement in performance. Rabbits treated with Adeno- β Gal did not show a significant difference in systolic function compared with saline-injected animals. This demonstrates that adenovirus

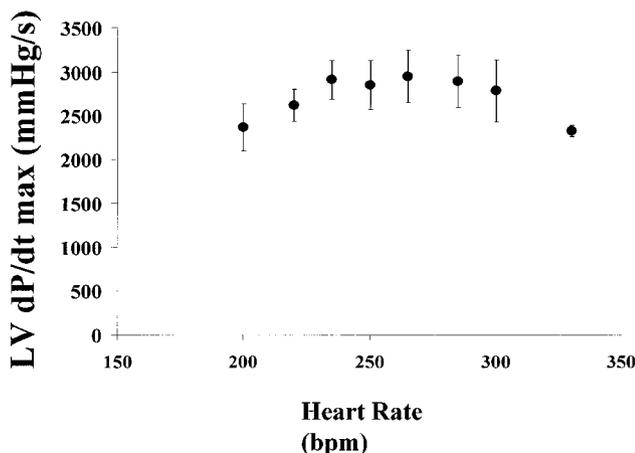


Figure 5. Effect of HR on LV dP/dt_{max} after atrial transvenous pacing (n=4).

TABLE 2. Summary of In Vivo LV Performance Before (Pre-Cath) and 6 Days After (Post-Cath) Adeno- β_2 AR Gene Delivery (n=4)

	Pre-Cath	Post-Cath (6 days)
dP/dt _{max} , mm Hg/s	2833.4±22	3593.7±307*
dP/dt _{min} , mm Hg/s	-2832.0±109	-2802.0±200
Isoproterenol-stimulated dP/dt _{max} , mm Hg/s	4646.4±242	5149.8±384†

* $P < 0.05$ vs Pre-Cath; † $P = 0.10$ vs Pre-Cath.

delivery, and transgene expression in general, does not depress baseline dP/dt_{max} . Interestingly, β_2 AR overexpression directed to the RV did not enhance LV performance. Although this result is not surprising, RV delivery of β_2 ARs may offer benefit in conditions of isolated right-sided dysfunction such as pulmonary hypertension.

We did observe a degree of myocardial injury with this model. In Adeno- β Gal controls and β_2 AR-treated rabbits, EDP was elevated and Adeno- β Gal-treated rabbits had depressed dP/dt_{min} values. Saline-injected controls did not show alterations of EDP or dP/dt_{min} . Overall, these data suggest that bolus delivery of adenovirus caused an increase in LV stiffness. Interestingly, animals that overexpressed the β_2 AR had less depression of dP/dt_{min} at 3 and 6 days, suggesting a superimposed lusitropic effect. Nevertheless, this apparent negative effect of intracoronary adenovirus injection needs to be examined further.

The present study highlights a number of challenges to in vivo cardiac gene therapy. First, myocardial delivery of the adenoviral transgene has been achieved in this percutaneous model, but whether the methods used here will be applicable to other viral vectors is not certain. The kinetics of adenovirus-mediated transfer in the intact coronary vasculature favor high coronary perfusion flow and pressure as well as enhanced permeability,²⁰ most of which can induce myocardial injury. We use a relatively large injection volume for individual coronary arteries, which we have found leads to significant improvement in gene transfer and transgene expression, consistent with the ex vivo kinetic studies of intracoronary adenoviral gene delivery.²⁰ Furthermore, the present study uses higher concentrations of virus than previously reported; however, transmural myocardial expression is also greater than in previous reports.¹⁰ Accordingly, a significant percentage of ventricular myocytes must be transfected to achieve a global functional impact.

The duration of transgene expression was not examined in this study. Work in our laboratory has documented that the first-generation vectors used yield expression that lasts for 1 to 2 weeks in vivo in the myocardium.^{9,16} However, we have clearly shown that adenovirus-mediated transgene delivery can alter the in vivo function of the heart for ≥ 1 week, and the use of this method can only improve in the future as vector technology advances to allow for less inflammatory vectors that also support longer-term expression. For example, long-term myocardial expression has recently been reported with adeno-associated virus.²¹ It will be of particular future interest to determine whether our delivery method will effectively deliver adeno-associated virus transgenes.

There are several implications of our experimental findings. The present study is among the first to demonstrate the feasibility of cardiac gene transfer by clinically relevant and available methods. Previous studies have used open thoracotomy and aortic cross-clamping,^{16,17} which would not be as widely useful to the 4 million patients with CHF. We have also demonstrated the ability to enhance baseline myocardial function in the otherwise normal LV. This improvement of baseline systolic function is consistent with previous findings in β_2 AR-overexpressing transgenic mice,^{12,13} including transgenic mice that had myocardial-targeted β_2 AR overexpres-

sion in the range reached by our adenovirus-mediated gene transfer.¹³ Thus, the possibility of genetic manipulation via the coronary circulation offers potential therapy to patients not amenable to traditional approaches to cardiomyopathy. Recent work has documented polymorphisms of the human β_2 AR gene, which result in dysfunctional G-protein coupling and predict poor prognosis in affected patients with CHF.²² Therefore, gene delivery of a normal β_2 AR gene might be particularly critical in this patient population.

The recent clinical evidence that failing cardiomyocytes have the capacity to reverse their remodeling and regain normal contractile function supports the hypothesis that molecular ventricular assistance, as shown here with β_2 AR overexpression, could serve as a novel form of therapy.^{23–25} This hypothesis is further strengthened by the finding that overexpression of β_2 ARs and an inhibitor of β -AR desensitization reverse abnormal signal transduction in cultured cardiomyocytes isolated from rabbits in CHF.¹⁵

A growing body of evidence supports the use of β -blockade in the treatment of heart failure.²⁶ Although our strategy would seem contradictory to this clinical experience, it may in fact be supported. Recent experimental work has shown that β -antagonist therapy with carvedilol is associated with a restoration of desensitized β -AR signaling that may be the mechanism of benefit of this agent in CHF.²⁷ Therefore, overexpression of functional β_2 ARs serves as an alternative method to restore β -AR signaling in failing myocardium.

In summary, the present study has demonstrated that subselective adenovirus-mediated delivery of a functional transgene is possible via the intact coronary circulation. Future studies will examine the consequences of acquired β_2 AR overexpression in models of CHF, and the present approach lends itself to that task. Furthermore, subselective gene delivery may allow the examination of new strategies for the management of hypertrophy, RV failure, and genetic cardiac maladaptation.

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