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# In Vivo Ventricular Gene Delivery of a $\beta$ -Adrenergic Receptor Kinase Inhibitor to the Failing Heart Reverses Cardiac Dysfunction

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**Background**—Genetic manipulation to reverse molecular abnormalities associated with dysfunctional myocardium may provide novel treatment. This study aimed to determine the feasibility and functional consequences of in vivo  $\beta$ -adrenergic receptor kinase ( $\beta$ ARK1) inhibition in a model of chronic left ventricular (LV) dysfunction after myocardial infarction (MI).

**Methods and Results**—Rabbits underwent ligation of the left circumflex (LCx) marginal artery and implantation of sonomicrometric crystals. Baseline cardiac physiology was studied 3 weeks after MI;  $5 \times 10^{11}$  viral particles of adenovirus was percutaneously delivered through the LCx. Animals received transgenes encoding a peptide inhibitor of  $\beta$ ARK1 (Adeno- $\beta$ ARKct) or an empty virus (EV) as control. One week after gene delivery, global LV and regional systolic function were measured again to assess gene treatment. Adeno- $\beta$ ARKct delivery to the failing heart through the LCx resulted in chamber-specific expression of the  $\beta$ ARKct. Baseline in vivo LV systolic performance was improved in Adeno- $\beta$ ARKct-treated animals compared with their individual pre-gene delivery values and compared with EV-treated rabbits. Total  $\beta$ -AR density and  $\beta$ ARK1 levels were unchanged between treatment groups; however,  $\beta$ -AR-stimulated adenylyl cyclase activity in the LV was significantly higher in Adeno- $\beta$ ARKct-treated rabbits compared with EV-treated animals.

**Conclusions**—In vivo delivery of Adeno- $\beta$ ARKct is feasible in the infarcted/failing heart by coronary catheterization; expression of  $\beta$ ARKct results in marked reversal of ventricular dysfunction. Thus, inhibition of  $\beta$ ARK1 provides a novel treatment strategy for improving the cardiac performance of the post-MI heart. (*Circulation*. 2001;103:1311-1316.)

**Key Words:** gene therapy ■ receptors ■ heart failure ■ signal transduction

Molecular abnormalities associated with and implicated in the pathogenesis of ventricular dysfunction and heart failure (HF) present appealing targets for cardiac gene therapy. In particular, genetic manipulation of myocardial  $\beta$ -adrenergic receptor ( $\beta$ -AR) signaling offers a powerful way to alter myocardial function and represents a potential target that has recently elicited much attention.<sup>1</sup>  $\beta$ -AR signaling abnormalities in the compromised and dysfunctional human heart have been well characterized and include a downregulation of  $\beta$ -ARs (specific for the  $\beta_1$ -AR subtype), uncoupling of second-messenger systems, and an upregulation of the  $\beta$ -adrenergic receptor kinase ( $\beta$ ARK1).<sup>2,3</sup>  $\beta$ ARK1 (also known as GRK2) is a member of the G-protein-coupled receptor kinase (GRK) family that phosphorylates agonist-occupied receptors, including cardiac  $\beta$ -ARs, triggering desensitization.<sup>4,5</sup> Recent evidence has revealed that enhanced

$\beta$ ARK1-mediated desensitization of myocardial  $\beta$ -ARs represents a maladaptive change in the failing heart,<sup>6</sup> and thus  $\beta$ ARK1 activity is a novel therapeutic target for potentially reversing ventricular dysfunction in cardiac disease states.

Importantly,  $\beta$ ARK1 has been found to be a critical regulator of myocardial function.<sup>5</sup> The expression and GRK activity of  $\beta$ ARK1 in the heart has been found to be significantly elevated in human<sup>3</sup> and animal models<sup>7-9</sup> of HF, hypertrophy,<sup>10</sup> and ischemia.<sup>11</sup> Studies in genetically engineered mice have demonstrated the utility of  $\beta$ ARK1 inhibition; expression of a  $\beta$ ARK1-inhibitory peptide has prevented HF as the result of the knockout of the muscle LIM protein gene.<sup>8</sup> The inhibitor of  $\beta$ ARK1 ( $\beta$ ARKct) is a peptide composed of the carboxyl-terminal 194-amino acid residues of  $\beta$ ARK1, which competes with endogenous  $\beta$ ARK1 for binding to the membrane-embedded  $\beta$ -subunits of activated

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heterotrimeric G-proteins, a process required for  $\beta$ ARK1 activation.<sup>5,12</sup>

Recently, catheter-based methods have enabled in vivo adenovirally mediated gene transfer to normal and hypertrophied myocardium.<sup>6,13,14</sup> These invasive methods in rats and rabbits have been developed to deliver transgenes globally to the beating heart in vivo. In a recent study, Adeno- $\beta$ ARKct was delivered globally to rabbit hearts at the time of the surgical induction of myocardial infarction (MI).<sup>6</sup> Interestingly, the acute inhibition of myocardial  $\beta$ -AR desensitization in the infarcted heart prevented the development of HF, demonstrating that the loss of  $\beta$ -AR coupling in the failing heart may not be solely an adaptive and protective mechanism but can contribute to the pathogenesis of HF.<sup>6</sup>

In this study, we have taken a unique approach in that the  $\beta$ ARKct transgene was not delivered to the heart until it was compromised in order to determine whether inhibition of  $\beta$ ARK1 activity in the failing heart could reverse physiological left ventricular (LV) dysfunction. We have recently demonstrated in vivo adenovirally mediated transgene delivery in a ventricular-specific manner through selective percutaneous coronary catheterization and gene delivery.<sup>15</sup> This technique was used in the present study, in which delivery of the  $\beta$ ARKct transgene into the left circumflex coronary artery (LCx) targets LV-specific expression and the untreated RV can serve as an internal control to test the efficacy of the transgene.  $\beta$ ARKct gene delivery to a dysfunctional heart is a critical step in validating  $\beta$ ARK1 inhibition as a potential therapy for HF.

## Methods

### Adenoviral Constructs

The construction, production, and purification of adenoviral constructs with a second-generation E1/E4-deleted, replication-deficient adenovirus have previously been described.<sup>6</sup> Three transgenes were used:  $\beta$ ARKct (Adeno- $\beta$ ARKct), the marker transgene  $\beta$ -Galactosidase (Adeno- $\beta$ Gal), and an empty viral construct (EV).

### Model of MI and Physiology

All animals received humane care, in compliance with guidelines prepared by the National Institutes of Health and according to protocols approved by Duke University. Adult male New Zealand White rabbits ( $\approx$ 3 kg) were used in the study. Animals underwent a thoracotomy and implantation of sonomicrometric crystals along the minor axis of the LV as described.<sup>16</sup> In 21 animals, a large marginal branch of the LCx was ligated as described,<sup>6</sup> whereas in 5 animals (the sham group), a prolene suture was passed around the vessel without ligation. Physiological assessment was made 3 weeks after MI to assess baseline cardiac function before gene delivery. A 2.5F micromanometer (Millar Inc) was placed into the LV cavity through the carotid artery under fluoroscopic guidance, and a 22-gauge angiocatheter was placed into the jugular vein. The sonomicrometric crystals and micromanometer were coupled to a PC-based data acquisition system (Physiological Systems Inc), and LV pressure (P) as well as segmental length (l) was obtained at baseline in spontaneously breathing animals. All hemodynamic data were derived from the average of 20 steady-state cardiac cycles.<sup>16</sup> Regional segmental length was used to determine systolic shortening (SS) as a measure of LV systolic function with the equation  $SS = (l_{ed} - l_{es})/l_{ed}$ , where  $l_{ed}$  and  $l_{es}$  represent end-diastolic and end-systolic length, respectively.<sup>16</sup> SS was then expressed as a percentage of pre-gene delivery values. Infarct size was measured as we have described<sup>6</sup> and did not significantly differ among treatment groups (data not shown). The mean infarct size on all animals was  $40 \pm 10\%$  of the LV.

### Intracoronary Gene Transfer

After the 3-week post-MI physiological assessment,  $5 \times 10^{11}$  total viral particles (tvp) of adenovirus in 2 mL of PBS was injected into the LCx after percutaneous catheterization, as we have previously described.<sup>15</sup> All rabbits received methylprednisolone (5.0 mg/kg IM per day) for 2 days after adenoviral delivery to limit the acute adenovirally mediated inflammatory response. Seven days after gene delivery (and 4 weeks after MI), cardiac function was studied in each rabbit as above.

### Determination of Myocardial Transgene Expression and $\beta$ -AR Signaling

To assess the efficacy of gene transfer to the infarcted rabbit heart, Adeno- $\beta$ Gal was delivered to the post-MI LCx as above. After excision of the heart, transverse cross sections of myocardium at the midpapillary level were obtained for histological analysis and X-gal staining as described.<sup>14,15</sup> To assess  $\beta$ ARKct transgene expression, ventricular RNA was isolated, and Northern blot analysis was performed by standard methods previously described.<sup>5,6</sup> Determination of cardiac  $\beta$ -AR density and membrane adenylyl cyclase (AC) activity were performed on myocardial sarcolemmal membranes with standard methods previously described.<sup>6,14,15</sup>

### Statistical Analysis

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

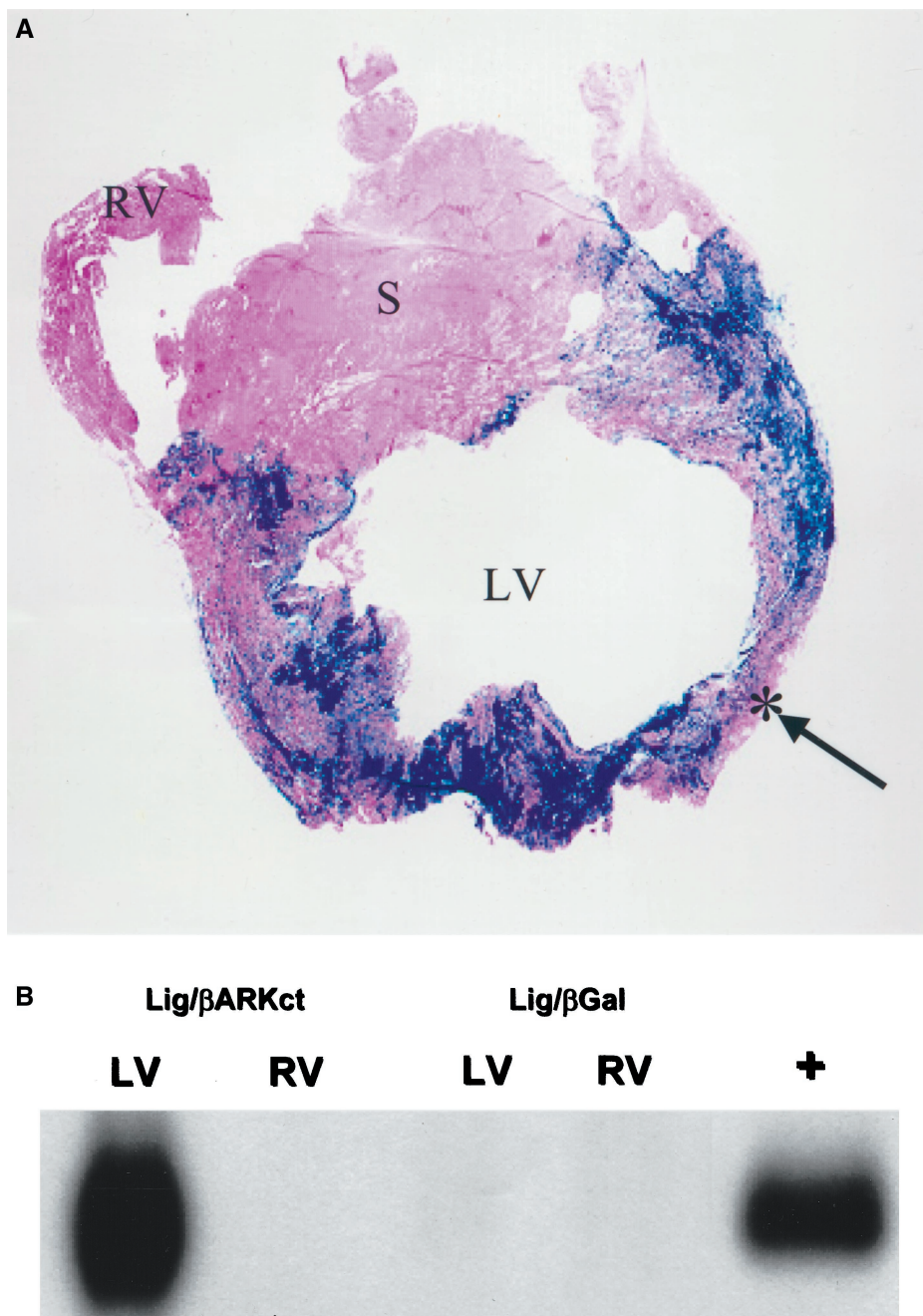
## Results

### In Vivo Intracoronary Delivery of Adenoviral Transgenes to Infarcted Rabbit Heart

LCx-mediated delivery of Adeno- $\beta$ Gal ( $5 \times 10^{11}$  tvp) to rabbits 3 weeks after MI resulted in robust expression of the transgene as determined by X-gal staining. Positive cardiomyocytes stained blue, and, as expected, were confined to the areas of the LV served by the vascular bed of the LCx (Figure 1A). Neither the septum nor the right ventricle (RV) stained blue, confirming a selective intracoronary delivery, and limited expression was found in the infarct zone (Figure 1A). Delivery of  $5 \times 10^{11}$  tvp of Adeno- $\beta$ ARKct through the LCx to infarcted rabbit hearts also displayed an LV-specific distribution of the transgene mRNA. A representative Northern blot demonstrating  $\beta$ ARKct transgene expression is shown in Figure 1B.

### Improvement of LV Dysfunction in Post-MI Hearts After Adeno- $\beta$ ARKct Delivery

The functional consequences of selective intracoronary delivery of Adeno- $\beta$ ARKct in post-MI rabbits was determined by sonomicrometry and micromanometry catheterization. Animals were initially studied 3 weeks after LCx ligation to assess baseline post-MI function. We have previously shown that 3 weeks after infarction is the time at which hemodynamic dysfunction and other signs of HF are clearly evident.<sup>6,7</sup> The function of the LV in post-MI rabbits 3 weeks after LCx ligation as assessed by SS with sonomicrometry crystals was profoundly depressed compared with rabbits that underwent sham operation. MI rabbits that were subsequently randomized to receive Adeno- $\beta$ ARKct or EV had a significant  $\approx 68\%$  decrease in LV SS compared with sham animals (Figure 2A). LV end-diastolic pressure (EDP) was signifi-



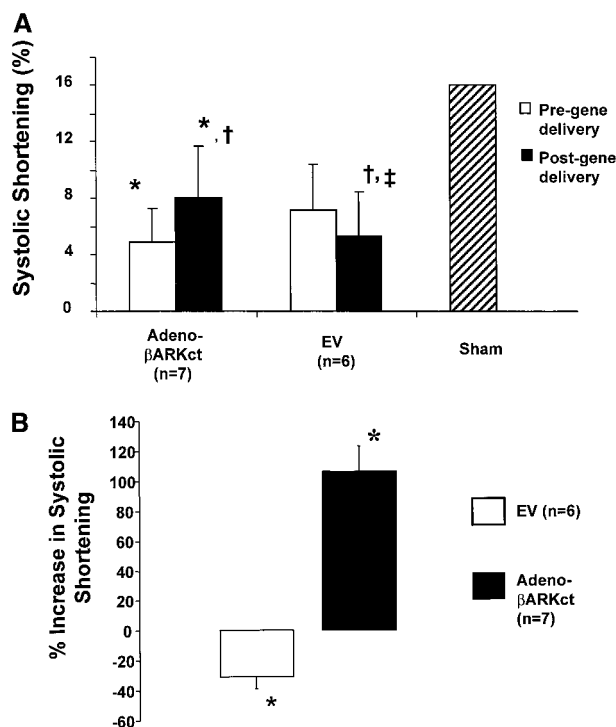
**Figure 1.** Transgene expression after LCx delivery. A, Representative whole mount of infarcted rabbit heart after treatment with  $5 \times 10^{11}$  tvp Adeno- $\beta$ Gal. Area of thinned and infarcted myocardium is seen in LV free wall and represents superior aspect of infarct (asterisk and arrow). B, Expression of  $\beta$ ARKct as confirmed by Northern blot analysis of RNA isolated from treated rabbit hearts.

cantly elevated in MI animals compared with sham (data not shown), as has been previously described in this model.<sup>6,7</sup>

MI rabbits with significant regional and global LV dysfunction were treated by LCx catheterization with  $5 \times 10^{11}$  tvp of Adeno- $\beta$ ARKct or EV and were allowed to progress for 1 more week. Regional LV function was then measured, and Adeno- $\beta$ ARKct-treated rabbits showed marked and significant improvement in LV SS compared with values obtained just before gene delivery (Figure 2A). This near 100% improvement in LV SS was not seen in EV-treated MI rabbits. In fact, these rabbits displayed a significant decrease in LV SS 1 week after gene delivery (Figure 2B). In a

separate subset of 3-week postinfarcted rabbits ( $n=5$ ), 2D echocardiography was performed<sup>6</sup> before and 1 week after Adeno- $\beta$ ARKct delivery. LV function as assessed by percent fractional shortening was significantly improved by  $\beta$ ARK1 inhibition (before,  $19.5 \pm 1\%$  versus after,  $23 \pm 2\%$ ,  $P < 0.05$ ,  $t$  test).

In vivo hemodynamics were evaluated by LV intracavitary pressure with micromanometer catheterization.<sup>6,7</sup> There was a small improvement in contractility as measured by LV  $dP/dt_{max}$ , after Adeno- $\beta$ ARKct delivery that did not reach statistical significance (Table). However, after  $\beta$ ARKct expression, peak systolic blood pressure was significantly

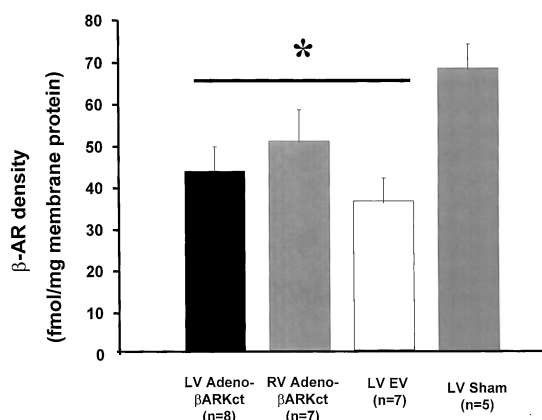


**Figure 2.** Regional LV SS determined by sonomicrometry. A, LV SS values in Adeno- $\beta$ ARKct-treated or EV-treated MI rabbits before and after gene delivery. All MI rabbit hearts had significant decrease in LV SS as compared with sham control rabbits. \* $P$ <0.05 vs sham; † $P$ <0.05 vs pre-gene delivery values; ‡ $P$ <0.05 vs Adeno- $\beta$ ARKct. B, Percent change in SS 1 week after gene delivery in EV-treated and Adeno- $\beta$ ARKct-treated animals. \* $P$ <0.05 vs pre-gene delivery values.

increased in the failing hearts after Adeno- $\beta$ ARKct treatment, which was also significantly greater than in EV-treated MI rabbits (Table). Interestingly, both LV EDP and HR did not significantly change after Adeno- $\beta$ ARKct delivery, whereas both of these parameters worsened (increased) in animals that received EV.

### Molecular $\beta$ -AR Signaling Changes Induced by $\beta$ ARKct Expression in Infarcted Heart

Three weeks after LCx ligation, a biventricular alteration of  $\beta$ -AR signaling was observed similar to that seen in other forms of HF. Samples from noninfarcted areas of the LV as well as RV



**Figure 3.**  $\beta$ -AR density values in MI and sham rabbit hearts.  $\beta$ -AR density was decreased after MI when compared with sham rabbits as measured 1 week after gene delivery. \* $P$ <0.05.

samples from MI rabbit hearts had a decrease in total  $\beta$ -AR density. Figure 3 contains this data. Sham animals had normal LV  $\beta$ -AR density of  $\approx$ 65 fmol per mg membrane protein, whereas LV and RV samples from 3-week post-MI rabbits that received Adeno- $\beta$ ARKct or EV had significantly downregulated  $\beta$ -ARs. In addition to  $\beta$ -AR density changes, post-MI rabbit hearts had elevated  $\beta$ ARK1 expression (data not shown) and uncoupled AC signaling (see below) compared with sham control hearts. These alterations in  $\beta$ ARK1 levels and  $\beta$ -AR signaling are consistent with those seen in our original study characterizing this rabbit HF model.<sup>7</sup> One week after Adeno- $\beta$ ARKct or EV delivery to the infarcted LV, there was no significant change in  $\beta$ ARK1 expression (data not shown).

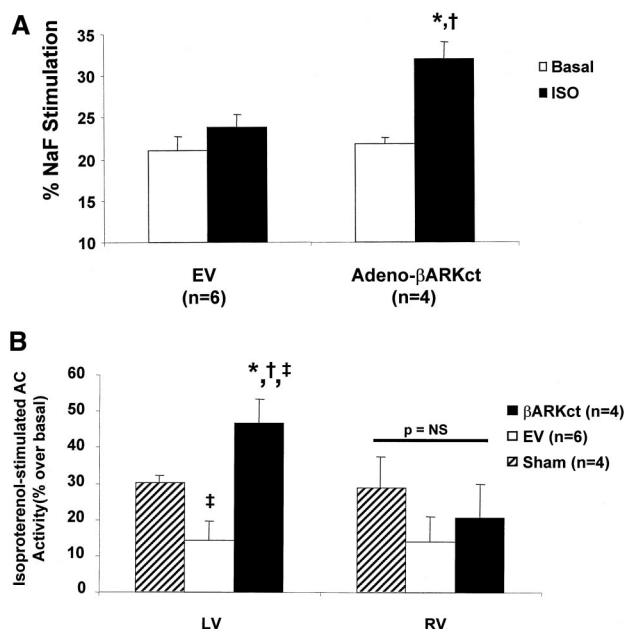
Despite no change in  $\beta$ -AR density among treated animals, desensitization of  $\beta$ -ARs in the infarcted LV was significantly reversed after Adeno- $\beta$ ARKct delivery. Shown in Figure 4A is baseline and isoproterenol-stimulated AC activity found in myocardial membranes isolated from post-MI hearts 1 week after EV or Adeno- $\beta$ ARKct delivery. As shown,  $\beta$ -AR-stimulated AC activity was minimal in the infarcted hearts treated with EV, demonstrating severe uncoupling, whereas hearts expressing the  $\beta$ ARK1 inhibitor had restored  $\beta$ -AR responsiveness (Figure 4A). In fact, as shown in Figure 4B, the  $\beta$ -AR responsiveness in  $\beta$ ARKct-treated LVs was greater than what we found in membranes from noninfarcted sham LVs. The AC data are also interesting

### Summary of In Vivo LV Performance as Determined by Micromanometer Catheterization

	Before Gene Delivery*		After Gene Delivery†	
	Adeno-EV	Adeno- $\beta$ ARKct	Adeno-EV	Adeno- $\beta$ ARKct
LV dP/dt <sub>max</sub> , mm Hg/s	2957±150	2818.7±259	2948±237	3045.3±247
LV dP/dt <sub>min</sub> , mm Hg/s	2463±74	2464±105	2356±80	2505.8±222
HR, bpm	245±8	232±11	281±2.8‡	251.5±19
LVEDP, mm Hg	3.8±0.5	5.0±0.8	8.3±0.8‡	6.6±2.3
LVSBP, mm Hg	66.8±1.8	69.4±2	67±2	75.8±3‡

Hemodynamic measurements were obtained at baseline, 3 weeks after LCx ligation,\* and again 1 week after delivery of  $5 \times 10^{11}$  tvp Adeno- $\beta$ ARKct or EV.†

‡ $P$ <0.05 vs pregene delivery.



**Figure 4.** Myocardial membrane AC activity. A, LV AC activity was significantly improved with isoproterenol (ISO) stimulation (as % NaF) after Adeno- $\beta$ ARKct treatment as compared with EV-treated hearts. There was no significant difference in basal AC activity.  $^*P < 0.05$  vs Adeno- $\beta$ ARKct baseline;  $^\dagger P < 0.05$  vs EV+ISO. B, Ventricular-specific  $\beta$ -AR-stimulated responsiveness was determined and expressed as percent increase from baseline.  $^\ddagger P < 0.05$  vs sham LV;  $^*P < 0.05$  vs EV-LV;  $^\dagger P < 0.05$  vs Adeno- $\beta$ ARKct RV.

from the point of view that they clearly illustrate the LV-selective gene targeting of the  $\beta$ ARKct caused by LCx-mediated delivery. As shown in Figure 4B, the  $\beta$ -AR responsiveness in RV membranes is not restored, as in the LV. The EV-treated infarcted hearts have a similar loss of isoproterenol-stimulated AC activity in both the RV and LV compared with sham values, demonstrating global loss of  $\beta$ -AR function in these MI rabbit hearts (Figure 4B).

### Discussion

This study reports two novel findings. The first is that in vivo percutaneous intracoronary gene delivery to the infarcted heart is feasible. Moreover, this gene delivery method can specifically target the failing LV. Second, we have demonstrated that adenovirally mediated myocardial delivery of the  $\beta$ ARKct transgene reverses  $\beta$ -AR signaling abnormalities and LV systolic dysfunction after MI. We have clearly demonstrated that a percutaneous delivery system, selectively catheterizing a coronary artery, can effectively deliver adenoviral transgenes to the failing heart after MI.

Importantly, in our model, gene delivery was accomplished through the LCx 3 weeks after the ligation of a marginal branch of the same artery. Transgene expression 1 week later was robust and widespread throughout the noninfarcted area of the LV. It is important to emphasize that the model used in this study does not completely represent ischemic heart disease as seen clinically. It is limited in that a single artery is ligated with a variable amount of infarcted myocardium, resulting in a range of LV dysfunction. However, we have

previously demonstrated that this model recapitulates the biventricular signaling abnormalities seen in HF.<sup>7</sup> Furthermore, the power of the study design is that each animal serves as its own control, with an untreated RV as an additional source for comparison in individual animals.

Biochemically, the expression of  $\beta$ ARKct in the LV improved  $\beta$ -AR signaling in the treated LV but not in the RV, which is a powerful demonstration of the ventricular specificity of our gene delivery methodology. In addition, LV AC activity in the Adeno- $\beta$ ARKct-treated animals was significantly higher than in EV-treated control rabbits, indicating that  $\beta$ -AR desensitization was attenuated as a consequence of  $\beta$ ARKct expression. Interestingly,  $\beta$ ARKct transgene expression did not alter  $\beta$ ARK1 levels between the LV and RV of Adeno- $\beta$ ARKct-treated rabbits. This suggests that expression of  $\beta$ ARKct at 1 week does not reverse the central neurohormonal or local mechanical stimulus responsible for  $\beta$ ARK1 upregulation. Importantly, it provides further evidence that the improvement in AC activity and in vivo LV function is due to active inhibition of  $\beta$ ARK1 by the  $\beta$ ARKct rather than a relative decrease in  $\beta$ ARK1 expression. Of course, because the  $\beta$ ARKct acts through  $G\beta\gamma$  inhibition, other signaling events mediated by  $G\beta\gamma$  may also contribute to the positive therapeutic effects seen in HF. Moreover, other receptor systems that are targets for  $\beta$ ARK1, in addition to  $\beta$ -ARs, may be involved.

We used both sonomicrometry and micromanometry to assess LV function in vivo in this small-animal model. Adeno- $\beta$ ARKct-treated animals had significant improvements in sonomicrometrically derived measures of LV systolic function. Moreover, there was a trend toward increased LV  $dP/dt_{max}$ . Although all of the parameters are load-sensitive, the regional improvement in function as demonstrated by SS suggests a contractile benefit of  $\beta$ ARKct expression. In fact, a significant decrease in LV SS was seen in control animals treated with the EV, suggesting an underlying decline in function secondary to catheterization or progression of dysfunction after 1 week of adenovirus delivery. The use of LV  $dP/dt$  as a measure of global LV function may be limited by the variation in infarct size and may be more sensitive to loading conditions in this model than in normal myocardium. LV EDP did not significantly change in Adeno- $\beta$ ARKct-treated animals compared with EV-treated control rabbits, in which it increased further between 3 and 4 weeks after MI. This demonstrates that in MI rabbits not treated with the  $\beta$ ARKct, LV dysfunction is progressing.

It bears mentioning that ventricular failure involves a myriad of receptor systems and abnormalities. It is unlikely that a single transgene can reverse all of the dysfunction and completely rescue the failing heart. However, of paramount importance is finding an efficacious transgene that is safe and does not ultimately damage myocardium. Perhaps more importantly, our catheter-based technique lends itself to other models of failure and hypertrophy and other intriguing questions may be answered with this technology. Thus, in addition to targeting myocardial  $\beta$ -AR signaling through  $\beta$ ARK1 inhibition, as in this study, or exogenously increasing  $\beta_2$ -AR density,<sup>14,15</sup> other worthwhile gene targets exist. The most promising appear to be manipulating myocardial  $Ca^{2+}$

handling through the sarcoplasmic reticulum ATPase<sup>13</sup> or phospholamban.<sup>18</sup> An additional target that has recently emerged is manipulation of K<sup>+</sup> channels in the cardiac sarcolemma, in attempts to alter repolarization abnormalities present in the failing heart.<sup>19</sup>

Several lines of evidence point toward the actions of  $\beta$ ARK1 being critically involved in the pathogenesis of HF. These include the fact that myocardial  $\beta$ ARK1 expression is elevated in chronic human HF,<sup>3</sup> contributing to the desensitization and downregulation of cardiac  $\beta$ -ARs.<sup>2,3</sup> Because  $\beta$ ARK1 expression and activity in the heart is also elevated in several animal models of disease,<sup>6–11</sup> we have recently used transgenic mice with myocardium-specific expression of  $\beta$ ARKct to further characterize the role of  $\beta$ ARK1 in heart disease. The potential therapeutic usefulness of  $\beta$ ARK1 inhibition was demonstrated in the prevention of HF in a genetic mouse model of cardiomyopathy.<sup>8</sup> This was accomplished by cross-breeding of the  $\beta$ ARKct transgenic mouse with a mouse HF model induced by “knockout” of the muscle LIM protein gene.<sup>8</sup> Before the current study, adenovirally mediated transfer of the  $\beta$ ARKct transgene to failing cardiomyocytes isolated from rabbits in HF resulted in the reversal of  $\beta$ -AR signaling abnormalities.<sup>17</sup> In addition, we have recently delivered Adeno- $\beta$ ARKct to rabbit hearts at the time of LCx ligation and MI and found that  $\beta$ ARK1 inhibition prevented the  $\beta$ -AR signaling abnormalities present acutely after MI and thereby significantly delayed the development of HF.<sup>6</sup>

This study provides unique insight into one approach for therapeutic cardiac gene therapy. Adenoviral vectors encoding  $\beta$ ARKct have allowed us to test the hypothesis that genetic manipulation of  $\beta$ -adrenergic signaling in adult myocardium may improve systolic function in the setting of acquired LV dysfunction. No reports have previously examined this question. Our model demonstrates that noninfarcted yet dysfunctional myocardium may be rescued at a molecular level. Thus,  $\beta$ ARK inhibition may represent a novel form of molecular ventricular assistance.

The fact that  $\beta$ -AR signaling is severely impaired in the failing heart no doubt provokes hyperactivity of the sympathetic nervous system; thus, a pathological cycle is perpetuated. Our hypothesis is that by relieving a brake on the system (ie,  $\beta$ ARK1), cardiac function can be improved, which can also chronically lead to the dampening of sympathetic overdrive, thus reversing the vicious cycle. Therefore, unlike  $\beta$ -agonists that can improve contractility acutely but further uncouple  $\beta$ -ARs through chronic stimulation, inhibition of  $\beta$ ARK1 begins to return  $\beta$ -ARs to a more normal state of signaling. This may not only lead to the acute improvement in cardiac performance but also can allow the compromised myocardium to recover from the chronic bombardment of catecholamines, which characterize the decompensated state. The present study, demonstrating the effectiveness of the  $\beta$ ARKct in reversing functional signaling abnormalities in the post-MI heart, adds weight to our recent study demonstrating that inhibition of  $\beta$ ARK1 at the time of LCx ligation can prevent acute  $\beta$ -AR signaling abnormalities and delay the development of HF. Both studies support our hypothesis that  $\beta$ ARK1 is an important HF target.<sup>20</sup> This novel therapeutic

strategy can be approached either through gene therapy with the  $\beta$ ARKct or through the development of pharmaceutical inhibitors of the  $\beta$ ARK1 G $\beta$  interaction.

### Acknowledgments

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### References

- Lefkowitz RJ, Rockman HA, Koch WJ. Catecholamines, cardiac “ $\beta$ ” adrenergic receptors, and heart failure. *Circulation*. 2000;101:1634–1637.
- Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and  $\beta$ -adrenergic receptor density in failing human hearts. *N Engl J Med*. 1982;307:205–211.
- Ungerer M, Bohm M, Elce JS, et al. Altered expression of  $\beta$ -adrenergic receptor kinase and  $\beta_1$ -adrenergic receptors in the failing human heart. *Circulation*. 1993;87:454–463.
- Inglese J, Freedman NJ, Koch WJ, et al. Structure and mechanism of the G protein-coupled receptor kinases. *J Biol Chem*. 1993;268:23735–23738.
- Koch WJ, Rockman HA, Samama P, et al. Cardiac function in mice overexpressing the  $\beta$ -adrenergic receptor kinase or a  $\beta$ ARK inhibitor. *Science*. 1994;268:1350–1353.
- White DC, Hata JA, Shah AS, et al. Preservation of  $\beta$ -adrenergic receptor signaling delays the development of heart failure after myocardial infarction. *Proc Natl Acad Sci U S A*. 2000;97:5428–5433.
- Maurice JP, Shah AS, Kypson AP, et al. Molecular  $\beta$ -adrenergic signaling abnormalities in failing rabbit hearts after infarction. *Am J Physiol*. 1999;276:H1853–H1860.
- Rockman HA, Chien KR, Choi DJ, et al. Expression of a  $\beta$ -adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. *Proc Natl Acad Sci U S A*. 1998;95:7000–7005.
- Cho MC, Rapacciuolo A, Koch WJ, et al. Defective  $\beta$ -adrenergic receptor signaling precedes the development of dilated cardiomyopathy in transgenic mice with calsequestrin overexpression. *J Biol Chem*. 1999;274:22251–22256.
- Choi, D-J, Koch WJ, Hunter JJ, et al. Mechanism of  $\beta$ -adrenergic receptor desensitization in cardiac hypertrophy is increased  $\beta$ -adrenergic receptor kinase. *J Biol Chem*. 1997;272:17223–17229.
- Ungerer M, Kessebohm K, Kronsbein K, et al. Activation of  $\beta$ -adrenergic receptor kinase during myocardial ischemia. *Circ Res*. 1996;79:455–460.
- Koch WJ, Inglese J, Stone WC, et al. The binding site for the  $\beta\gamma$  subunits of heterotrimeric G proteins on the  $\beta$ -adrenergic receptor kinase. *J Biol Chem*. 1993;268:8256–8260.
- Miyamoto MI, del Monte F, Schmidt U, et al. Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *Proc Natl Acad Sci U S A*. 2000;97:793–798.
- Maurice JP, Hata JA, Shah AS, et al. Enhancement of cardiac function after adenoviral-mediated in vivo intracoronary  $\beta_2$ -adrenergic receptor gene delivery. *J Clin Invest*. 1999;104:21–29.
- Shah AS, Lilly RE, Kypson AP, et al. Intracoronary adenovirus-mediated delivery and overexpression of the  $\beta_2$ -adrenergic receptor in the heart: prospects for molecular ventricular assistance. *Circulation*. 2000;101:408–414.
- Silvestry SC, Taylor DA, Lilly RE, et al. The in vivo quantification of myocardial performance in rabbits: a model for evaluation of cardiac gene therapy. *J Mol Cell Cardiol*. 1996;28:815–823.
- Akhter SA, Skaer CA, Kypson AP, et al. Restoration of  $\beta$ -adrenergic signaling in failing cardiac ventricular myocytes via adenoviral-mediated gene transfer. *Proc Natl Acad Sci U S A*. 1997;94:12100–12105.
- Eizema K, Fechner H, Bezstarosty K, et al. Adenovirus-based phospholamban antisense expression as a novel approach to improve cardiac contractile dysfunction: comparison of a constitutive viral versus an endothelin-1-responsive cardiac promoter. *Circulation*. 2000;101:2193–2199.
- Hoppe UC, Marban E, Johns DD. Molecular dissection of cardiac repolarization by in vivo Kv4.3 gene transfer. *J Clin Invest*. 2000;105:1077–1084.
- Iaccarino G, Lefkowitz RJ, Koch WJ. Myocardial G protein-coupled receptor kinases: implications for heart failure therapy. *Proc Assoc Am Physicians*. 1999;111:399–405.