Intracoronary Delivery of Adenovirus Encoding Adenylyl Cyclase VI Increases Left Ventricular Function and cAMP-Generating Capacity

N. Chin Lai, PhD; David M. Roth, PhD, MD; Mei Hua Gao, PhD; Suzanne Fine, MS; Brian P. Head, BA; Jian Zhu, BS; M. Dan McKirnan, PhD; Christopher Kwong, BS; Nancy Dalton, BA; Kazushi Urasawa, MD; David A. Roth, PhD; H. Kirk Hammond, MD

Background—We tested the hypothesis that intracoronary injection of a recombinant adenovirus encoding adenylyl cyclase type VI (AC VI) would increase cardiac function in pigs.

Methods and Results—Left ventricular (LV) dP/dt and cardiac output in response to isoproterenol and NKH477 stimulation were assessed in normal pigs before and 12 days after intracoronary delivery of histamine followed by intracoronary delivery of an adenovirus encoding lacZ (control) or AC VI (1.4×10^{12} vp). Animals that had received AC VI gene transfer showed increases in peak LV dP/dt (average increase of 1267±807 mm Hg/s; \( P<0.0002 \)) and cardiac output (average increase of 39±20 mL·kg^{-1}·min^{-1}; \( P<0.0001 \)); control animals showed no changes. Increased LV dP/dt was evident 6 days after gene transfer and persisted for at least 57 days. Basal heart rate, blood pressure, and LV dP/dt were unchanged, despite changes in cardiac responsiveness to catecholamine stimulation. Twenty-three hour ECG recordings showed no change in mean heart rate or ectopic beats and no arrhythmias. LV homogenates from animals receiving AC VI gene transfer showed increased AC VI protein content (\( P<0.0007 \)) and stimulated cAMP production (\( P<0.0006 \)), confirming transgene expression and function; basal LV AC activity was unchanged. Increased cAMP-generating capacity persisted for at least 18 weeks (\( P<0.0002 \)).

Conclusions—Intracoronary injection of a recombinant adenovirus encoding AC provides enduring increases in cardiac function. (Circulation. 2000;102:2396-2401.)

Key Words: receptors, adrenergic, beta  n gene transfer  n heart failure  n histamine  n myocardial contraction

We recently demonstrated that the amount of adenylyl cyclase (AC) is the primary limiting factor of a cell’s ability to generate cAMP in response to \( \beta \)-adrenergic receptor (\( \beta \)AR) stimulation. 1 We subsequently showed that transgenic mice with cardiac-directed expression of AC type VI (AC VI) have increased responses to catecholamine 2 and that when cardiac AC VI is overexpressed in a cardiomyopathic background, left ventricular (LV) function is improved 3 and life is prolonged (unpublished data).

However, there are enormous differences between the transgenic mouse paradigm and exogenous gene transfer to adult animals. For example, the use of a cardiac-directed promoter in transgenic mice can provide high-level transgene expression exclusively and ubiquitously in cardiac myocytes. These features are in stark contrast to exogenous gene transfer to adult animals, where the efficiency of gene transfer is limited, transgene expression not as robust, and cell specificity more difficult to obtain with commonly employed vectors.

This is an important issue scientifically, but it also has potential clinical implications. For example, if these impediments to successful gene transfer could be overcome, it is possible that clinical dilated heart failure could be treated more effectively. Clinical application would require a method of gene transfer that is safe, effective, and preferably nonsurgical and that would have enduring effects on LV function. The goal of the present study was to determine if global LV function could be increased by intracoronary delivery of an adenovirus expressing AC VI.

Methods

A total of 63 pigs were used in accordance with the National Institutes of Health and institutional guidelines. Fifty-four animals (mean weight, 38±1 kg) underwent intracoronary delivery of a...
Figure 1. Adenovirus-mediated gene transfer. A and B, Transmural sections of LV (magnification: 30× in A and 200× in B) obtained 5 days after intracoronary delivery of adenovirus expressing nuclear localized lacZ (10^12 vp). Injection was preceded by intracoronary infusion of histamine (25 μg/min for 3 minutes). Gene transfer was easy to detect (blue color) and was more extensive than that achieved without histamine. C, Effects of varying doses of intracoronary adenovirus encoding ACVI on LV cAMP generation (100 μmol/L NKH477) 12 days after gene transfer. Assays were conducted on LV membranes from each group simultaneously. In absence of precedent intracoronary histamine, there was a decrease in LV cAMP levels in animals that were administered histamine. Bars indicate mean ± SEM. Sample size is indicated on each bar.

We next instrumented 18 pigs for studies of LV contractile function, which were performed 12 ± 1 days after gene transfer (ACVI, n = 10; lacZ, n = 8). To determine the onset and duration of the physiological effects, studies were conducted in a subset of these animals at earlier (6 days) and later (30 and 60 days) time points. In addition, 8 pigs were used to determine whether intracoronary delivery of adenovirus in doses of 5 × 10^12 vp (n = 3) and 1.4 × 10^12 vp (n = 5), without precedent histamine, would also result in an increased LV cAMP-generating capacity.

Finally, 23 uninstrumented animals (ACVI, n = 16; lacZ, n = 7) were used to determine the effects of ACVI gene transfer on cAMP production in LV membranes at 4, 8, 12, and 18 weeks after gene transfer. Twenty-three hour ECG recordings (Holter Recorder Model 461A, Del Mar Medical Systems) were obtained in 17 of these pigs (ACVI, n = 11; lacZ, n = 6) before and 10 ± 1 days after gene transfer.

Instrumentation
LV pressure transducers (Konigsberg Instruments, Inc.), pulmonary arterial or aortic flow probes (Transonic Inc.), ECG wires, and left atrial, aortic, and pulmonary arterial catheters were placed in 18 pigs. Then, 19 ± 2 days later, hemodynamic measurements and assessment of LV global function were performed before and during agonist infusions.

Gene Transfer
E1-deleted, replication-incompetent adenovirus vectors encoding murine ACVI or nuclear-tagged bacterial β-galactosidase (lacZ), driven by a cytomegalovirus promoter, were generated by homologous recombination, as previously described.1 For instrumented animals, gene transfer was performed 3 ± 1 days after completion of pharmacological tests. All animals except those enrolled in the no-histamine studies received a 3-minute intracoronary infusion of histamine diphosphate (25 μg/min; Sigma) into each of the 3 major coronary branches, followed by a total dose of 1.4 × 10^12 vp adenovirus (left anterior descending, 0.7 × 10^12 vp; left circumflex, 0.4 × 10^12 vp; right coronary artery, 0.3 × 10^12 vp). Animals that were not given precedent intracoronary histamine received 1.4 × 10^12 vp (lacZ, n = 5; ACVI, n = 5), 5 × 10^12 vp (ACVI, n = 3), or no gene transfer (n = 9). In instrumented animals, hemodynamics and LV global function before and during agonist infusions were repeated 12 ± 1 days after gene transfer. Pigs were killed and tissues were collected 12 ± 2 days after gene delivery for histopathological and biochemical studies. Eighteen animals that had received ACVI gene transfer were killed 4 (n = 4), 8 (n = 6), 12 (n = 4), and 18 (n = 4) weeks after gene transfer. Five additional animals that had received lacZ gene transfer were used as controls for these studies.

LV Contractile Function
Echocardiographic assessments (Hewlett-Packard Sonos 1000) were obtained in conscious animals2 to determine the effects of gene transfer on LV size and basal function. Two or 3 tests conducted on separate days were averaged before and after gene transfer. Basal LV pressure, cardiac output, and heart rate were recorded before and after administration of the glycopyrrolate (0.07 mg/kg) used to remove vagal tone. The response after graded intravenous bolus injections of (-)-isoproterenol (0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 μg/kg) was assessed. Subsequently, LV pressure was recorded during the intravenous administration of 3, 10, and 30 μg/kg NKH477, a water-soluble forskolin derivative (Nippon Kayaku, Tokyo, Japan). Data analyses were blinded.

AC Assays
Methods were modified from Salomon, as previously reported.1 NKH477 (0.1 to 100 μmol/L) was used to stimulate cAMP production to assess AC function.

ACVI Protein Content
LV homogenates were centrifuged through a 5% to 35% discontinuous sucrose gradient. Fractions containing caveolin-3 were isolated4 and concentrated with a size-exclusion centrifugal filter (Amicon Centrprep). Then, an anti-ACVI primary antibody (Santa Cruz Biotechnology) and a secondary antibody coupled to horseradish peroxidase were used to detect ACVI from these fractions (1.5 μg of protein). A glutathione S-transferase-human-ACVI fusion protein was loaded with each sample to ensure that equal amounts of protein were added in each lane. The intensity of the ACVI bands was measured by densitometry (Storm 840, Molecular Dynamic).

LV βBAR, G Protein, and G Protein Receptor-Coupled Kinase-2 Content
Crude LV membrane homogenates were used in immunoblotting studies to determine the protein contents of β2 and β2-adrenergic receptors and of Gαs, Gαi2, and G protein receptor-coupled kinase-2 (GRK2), using methods previously described.2

Assessment of Inflammation and Fibrosis
Hematoxylin and cosin and Masson’s trichrome stains were used to detect inflammatory cell infiltrates, cell necrosis, and fibrosis in
samples from the lung, liver, and LV in uninstrumented animals that had received gene transfer of ACVI at 1.4×10^{12} vp (with histamine) killed 4 weeks after gene transfer (n=4) and 5.0×10^{12} vp (no histamine) killed 2 weeks after gene transfer (n=3). Five animals that had not received adenovirus were used as controls. Histological analysis was blinded.

### Data Analysis
Differences in hemodynamic measurements and in cAMP production were assessed by ANOVA. Protein contents were compared using Student’s t test for paired or unpaired data (2-tailed).

### Results

#### Gene Transfer and Histamine Pretreatment
Figure 1 indicates that there is a relationship between the amount of adenovirus encoding ACVI delivered and the effect on LV cAMP generation measured 12 days later and that the effect is increased by precedent intracoronary histamine infusion. In the absence of precedent intracoronary histamine, 5×10^{12} vp (but not 1.4×10^{12} vp) of adenovirus encoding ACVI increased LV cAMP above control values. In contrast, when intracoronary delivery of 1.4×10^{12} vp of an adenovirus encoding ACVI was preceded by histamine infusion, LV cAMP production increased 3-fold (versus lacZ); production increased 2.8-fold over what was seen when the same dose had been administered without histamine.

#### LV Contractile Function
LV dimensions were unaltered by gene transfer (Table 1), but basal fractional shortening was mildly increased after ACVI gene transfer. Basal hemodynamics, LV dP/dt, and cardiac output were unaffected by gene transfer (Table 1). During adrenergic stimulation, pigs that received lacZ gene transfer showed no differences in LV dP/dt or cardiac output response compared with their responses before gene transfer (Figure 2). In contrast, ACVI gene transfer was associated with increased LV dP/dt and cardiac output during isoproterenol stimulation, with maximum gains of 1267±807 mm Hg/s in peak LV dP/dt (P<0.0002) and of 39±20 mL·kg⁻¹·min⁻¹ in cardiac output (P<0.0001) versus the pretransfer values. NKH477 stimulation was associated with a maximum gain of 2515±890 mm Hg/s in peak LV dP/dt. Changes in LV dP/dt were evident 6 days after gene transfer and persisted for at least 57 days (Figure 3D), which was the time of final testing.

#### ECG Recordings
Gene transfer (Table 1) did not alter mean heart rate over a 23±0.5-hour period. A normal diurnal variation in heart rate was evident, with no difference between groups. Supraventricular and ventricular tachycardias were not present. Isolated rare unifocal premature ventricular contractions

### Table 1. Basal Measurements Before and 12±1 Days After Intracoronary Delivery of Adenovirus Expressing ACVI or lacZ

<table>
<thead>
<tr>
<th></th>
<th>Pre-Transfer</th>
<th>Post-Transfer</th>
<th>P</th>
<th>Pre-Transfer</th>
<th>Post-Transfer</th>
<th>P</th>
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<tr>
<td></td>
<td>2.6±0.1 (n=12)</td>
<td>2.7±0.1 (n=12)</td>
<td>0.29</td>
<td>2.6±0.1 (n=14)</td>
<td>2.6±0.1 (n=14)</td>
<td>0.78</td>
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<td>ESD, cm</td>
<td>4.3±0.1 (n=12)</td>
<td>4.4±0.2 (n=12)</td>
<td>0.22</td>
<td>4.2±0.1 (n=14)</td>
<td>4.4±0.1 (n=14)</td>
<td>0.16</td>
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<tr>
<td>RR, bpm</td>
<td>108±6 (n=8)</td>
<td>108±6 (n=8)</td>
<td>0.92</td>
<td>104±6 (n=10)</td>
<td>109±7 (n=10)</td>
<td>0.35</td>
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<tr>
<td>LV dP/dt, mm Hg/s</td>
<td>2952±211 (n=8)</td>
<td>2654±191 (n=8)</td>
<td>0.24</td>
<td>2493±257 (n=10)</td>
<td>2554±183 (n=10)</td>
<td>0.76</td>
</tr>
<tr>
<td>Cardiac output, mL·kg⁻¹·min⁻¹</td>
<td>108±11 (n=7)</td>
<td>115±16 (n=7)</td>
<td>0.47</td>
<td>112±10 (n=7)</td>
<td>133±10 (n=7)</td>
<td>0.13</td>
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<tr>
<td>AO, mm Hg</td>
<td>119±6 (n=8)</td>
<td>112±5 (n=8)</td>
<td>0.41</td>
<td>116±5 (n=10)</td>
<td>114±3 (n=10)</td>
<td>0.69</td>
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<td>PA, mm Hg</td>
<td>21±2 (n=8)</td>
<td>22±1 (n=8)</td>
<td>0.48</td>
<td>23±1 (n=10)</td>
<td>24±1 (n=10)</td>
<td>0.19</td>
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<tr>
<td>LA, mm Hg</td>
<td>10±1 (n=8)</td>
<td>11±1 (n=8)</td>
<td>0.57</td>
<td>12±1 (n=9)</td>
<td>13±1 (n=9)</td>
<td>0.30</td>
</tr>
<tr>
<td>23-Hour mean HR, bpm</td>
<td>109±5 (n=6)</td>
<td>102±5 (n=6)</td>
<td>0.16</td>
<td>105±5 (n=11)</td>
<td>98±6 (n=11)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n). ESD indicates end-systolic dimension; EDD, end-diastolic dimension; FS, fractional shortening; HR, heart rate; AO, aortic pressure; LA, left atrial pressure; and PA, pulmonary artery pressure.

### Figure 2. Cardiac function. The y-axes of graphs represent mean values obtained 12±1 days after gene transfer minus mean values obtained before gene transfer. A y value near zero indicates that gene transfer had no effect (lacZ, C), whereas a positive y value indicates that gene transfer was associated with increased responsiveness (ACVI, ●). A, ACVI gene transfer was associated with increased LV dP/dt (versus pretransfer value) through a wide range of concentrations of infused isoproterenol (ACVI, n=10; lacZ, n=8). B, ACVI gene transfer was associated with increased LV peak negative dP/dt, indicating increased relaxation and diastolic function (ACVI, n=10; lacZ, n=8). C, Cardiac output (CO) stimulated by isoproterenol was increased after ACVI gene transfer through a wide range of concentrations of infused isoproterenol (ACVI, n=7; lacZ, n=7). D, Direct stimulation of ACVI (NKH477) also showed increased contractile responsiveness in animals after ACVI gene transfer (ACVI, n=7; lacZ, n=5). Error bars denote 1 SEM. P value is from 2-way ANOVA.
ANOVA).

However, AC VI gene transfer was associated with a 13% increase in cAMP-generating capacity 2 weeks after gene transfer (Figure 3C). These changes did not occur after intracoronary delivery of 1.4×10^{12} vp (n=3). We did not examine the liver and lung for the presence of AC VI transgene expression.

**Discussion**

Our data indicated that 12 days after AC VI gene transfer, the heart’s contractile response to βAR stimulation increased in parallel with increased LV AC VI protein content and cAMP-generating capacity. These changes did not occur after intracoronary delivery of an adenovirus encoding lacZ. Changes

### LV βAR, G Protein, and GRK2 Content

No differences in β_{1}- or β_{2}-adrenergic receptors, Gαs, or Gαi2 were detected in LV membranes from animals that had received AC VI gene transfer 12 days before testing (Table 2). However, AC VI gene transfer was associated with a 13% increase in immunodetectable GRK2 in LV samples.

### Histopathology

No differences existed in inflammatory cell infiltrates, cell necrosis, or fibrosis in samples from the lung, liver, and LV of control animals (n=5) versus samples from animals that had received gene transfer of AC VI at 1.4×10^{12} vp (n=4) or 5.0×10^{12} vp (n=3). We did not examine the liver and lung for the presence of AC VI transgene expression.

#### TABLE 2. Data From Immunoblotting Conducted on LV Homogenates

<table>
<thead>
<tr>
<th></th>
<th>lacZ</th>
<th>AC VI</th>
<th>P</th>
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<tr>
<td>β_{1}-Adrenergic receptors</td>
<td>75±4</td>
<td>76±3</td>
<td>0.9</td>
</tr>
<tr>
<td>β_{2}-Adrenergic receptors</td>
<td>210±3</td>
<td>237±15</td>
<td>0.12</td>
</tr>
<tr>
<td>Gαs</td>
<td>51±1</td>
<td>54±1</td>
<td>0.19</td>
</tr>
<tr>
<td>Gαi2</td>
<td>92±6</td>
<td>81±12</td>
<td>0.43</td>
</tr>
<tr>
<td>GRK2</td>
<td>56±1</td>
<td>63±3</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Animals received intracoronary histamine (25 μg/min, 3 min) followed by intracoronary delivery of the adenovirus vector and were killed 12 days later. All values are densitometric units×10^{-3}. For all entries, n=4.
in LV contractility were observed 6 days after gene transfer and persisted for at least 57 days. Increased LV cAMP-generating capacity persisted for at least 18 weeks after a single intracoronary injection of adenovirus encoding ACβ1.

There was no alteration in basal LV function or basal AC activity. The LV content of β1- and β2-adrenergic receptors and of the stimulatory and inhibitory GTP-binding proteins (Gαs and Goi) was also unchanged. The small increase in GRK2 would not be expected to contribute to increased LV cAMP responsiveness, but it might mildly attenuate signal transduction. Therefore, the favorable effects on LV contractile function and cAMP generation were the result of increased transgene expression of ACβ1 in LV membranes. Gene transfer was not associated with histological abnormalities in the heart, liver, or lung, even at doses of 5×10^12 vp. This confirms the results of a previous study.4 We previously showed, using transgenic mice, the favorable effects of prolonged cardiac-directed AC expression2 and its effectiveness in treating experimental cardiomyopathy.3 The favorable alteration in global LV function after intracoronary delivery of an adenovirus encoding ACβ1 suggests that this strategy can be used as an alternative to transgenic mice, thereby circumventing problems associated with the transgenic mice paradigm. It also opens the door for possible future clinical applications.

We recently showed that intracoronary delivery of an adenovirus encoding an angiogenic gene improves regional myocardial contractile function in myocardial ischemia6 and in heart failure.8 In the current study, we used intracoronary infusion of histamine followed by delivery of the adenovirus, because the secreted transgene has a ubiquitous gene transfer because the secreted transgene has a second effect, namely, the elaboration of new blood vessels. In contrast, the present objective was to upregulate an integral membrane protein (ACβ1), and we presumed that the degree of increase in LV function would directly reflect the extent of gene transfer. The ability of intracoronary gene transfer to achieve global alterations in LV function was somewhat surprising, because the extent of gene transfer obtained after intracoronary delivery of an adenovirus is estimated to be <30%.7 Agents that increase vascular permeability reportedly increase the extent of gene transfer conferred by intracoronary delivery of an adenovirus in ex vivo–perfused hearts.8 In the current study, we used intracoronary infusion of histamine followed by delivery of the adenovirus, because our initial pilot studies demonstrated substantial gene transfer in the heart using this technique (Figure 1). We then determined that intracoronary histamine before intracoronary adenovirus infusion increased the effect on LV cAMP generation by nearly 3-fold (Figure 1). Intracoronary histamine infusion may increase intracoronary gene transfer through its effects on vascular permeability or by increased transvascular transport across endothelial cells.9

Measurement of LV contractile function required the maintenance of a pressure transducer in the LV chamber. This device rarely gives suitable data for longer than 2 months; therefore, we were unable to obtain data regarding LV contractile function beyond 57 days after gene transfer (Figure 3D). It is noteworthy that peak LV dP/dt in response to isoproterenol is as high 57 days after gene transfer as it was 12 days after gene transfer, suggesting that the physiological effect does not decay over this interval. The persistent elevation in LV cAMP-generating capacity throughout the 18-week study (Figure 3C) may indicate that LV contractile function may be increased this long as well.

These persistent changes in transgene expression and function are longer than expected after adenovirus-mediated gene transfer.7 Perhaps this reflects a long biological half-life of the ACβ1 protein, although we can find nothing in the literature regarding the turnover rate of newly synthesized ACβ1. Other considerations are that the cardiac myocyte is postmitotic, so a dilutional effect of cell division on the proportion of cells expressing the transgene is not a factor. Furthermore, the absence of myocardial inflammation, which we confirmed by the absence of CD4-and CD8-expressing T lymphocytes in previous studies,4 might result in more prolonged gene expression than observed in other settings that are associated with an inflammatory response.

Sustained stimulation of the heart and provocation of arrhythmias are reasonable concerns with amplification of cardiac adrenergic responsiveness. However, there was no increase in basal cardiac output, LV dP/dt, or blood pressure associated with ACβ1 gene transfer. Likewise, basal cAMP was unchanged, which is consistent with previous findings in transgenic mice with cardiac-directed overexpression of ACβ12 and in adenovirus-mediated ACβ1 gene transfer in cultured cardiac myocytes.1 Finally, 23-hour ECG recordings showed unchanged mean heart rates and no arrhythmias after ACβ1 gene transfer. Provocation of arrhythmia may be more likely in the setting of heart failure, but cardiac overexpression of ACβ1 is associated with improved heart function1 and reduced mortality (unpublished data), suggesting that ACβ1 treatment does not provoke life-threatening arrhythmias in experimental heart failure.

Three studies have used direct or indirect intracoronary gene transfer to alter global LV function.10–12 These studies substantiate the effectiveness of an intracoronary injection of recombinant adenovirus to change cardiac function, confirming in our initial report in 1996.4 Weig et al10 used a coronary infusion of low calcium and serotonin followed by an adenovirus encoding V2 vasopressin receptors (10^10 pfu). This was associated with a 20% increase in LV dP/dt response at 3 doses of desmopressin acetate in anesthetized animals in studies performed an unspecified time after gene transfer. However, this was an unblinded study in anesthetized rabbits, and LV dP/dt was only 40% of normal because of the negative inotropic effects of the anesthetic agents that were used. Maurice et al11 used adenosine pretreatment in rabbits followed by indirect intracoronary injection of an adenovirus encoding β2-adrenergic receptors (10^12 vp). They observed increased LV dP/dt in anesthetized animals in response to 4 doses of isoproterenol (the mean increase was 13% versus lacZ-treated animals at 6 days and 20% at 21 days). However, cardiac overexpression of βAR13,14 or Gs15 provides sustained adrenergic stimulation with undesirable consequences, including increased mortality, in experimental cardiomyopathy.14 cardiac fibrosis,13,15 and cardiomyopathy.13,15 Hajjar et al12 used thoracotomy followed by indirect intracoronary delivery in rats of an adenovirus encoding phospholamban and reported a 39% reduction in LV dP/dt.
when anesthetized animals were studied 2 days later. However, the effect was gone by 5 days. In these studies, the amount of adenovirus delivered to the rat heart was 30- to 90-fold higher per gram of rat heart than what we delivered to the pig heart. This may explain the cardiac inflammation seen in the rat heart, which could cause the short-lived effects.

A number of clinical trials of inotropic agents that increase cAMP levels in the failing heart indicate that such a strategy is contraindicated. However, the agents studied in these trials resulted in sustained elevations of cAMP via stimulation of the βAR with dobutamine infusion or by blocking cAMP breakdown with the phosphodiesterase inhibitor milrinone. It may be the unrelenting stimulation of the heart from sustained cAMP elevations that led to deleterious consequences in these clinical trials. In contrast, with ACVI gene transfer, there is no change in basal levels of cAMP in cardiac myocytes.1–3 The beneficial effects of ACVI overexpression in the setting of experimental heart failure support the possibility that this mode of treatment could improve LV contractile responsiveness in clinical heart failure without detrimental effects. Clearly, tests in animal models are not conclusive, but the data justify further evaluation of this strategy in clinical heart failure.

In conclusion, intracoronary delivery of an adenovirus expressing ACVI increases global LV contractile function in normal pigs. AC overexpression provided increased recruitable adrenergic responsiveness, resulting in increased LV contractile function. This effect was present 6 days after gene transfer and persisted for at least 57 days. Increased function was associated with increased LV ACVI protein content and an increased ability of cardiac myocytes to generate cAMP, an effect that persisted for at least 18 weeks. There was no evidence of sustained adrenergic activation or arrhythmias. These data, together with previous reports, suggest that this approach may be suitable for the treatment of clinical heart failure.

Acknowledgments

Supported by the Department of Veteran’s Affairs (Merit award to H.K.H. and Research Career Development Award to D.M.R.), grants from the National Institutes of Health (NIH1P50HL-53773-01 and 2P50HL-53773-06 to H.K.H.), and Collateral Therapeutics, Inc. We thank Matthew Spellman, Muriel Spooner, Xin Guo, Gary Anderson, Mona Logan, and Bob Worth for technical assistance; Dr Joe Voland for histological review; Nippon Kayaku for providing NKH477; and Dr Tamsin Lisa Kelly for reviewing the manuscript.

References

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Circulation. 2000;102:2396-2401
doi: 10.1161/01.CIR.102.19.2396

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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