Increased Cardiac Adenylyl Cyclase Expression Is Associated With Increased Survival After Myocardial Infarction

Toshiyuki Takahashi, MD, PhD; Tong Tang, PhD; N. Chin Lai, PhD; David M. Roth, PhD, MD; Brian Rebolledo, BA; Miho Saito, MD; Wilbur Y.W. Lew, MD; Paul Clopton, MS; H. Kirk Hammond, MD

Background—Cardiac-directed expression of adenylyl cyclase type VI (ACVI) in mice results in structurally normal hearts with normal basal heart rate and function but increased responses to catecholamine stimulation. We tested the hypothesis that increased left ventricular (LV) ACVI content would increase mortality after acute myocardial infarction (MI).

Methods and Results—Transgenic mice with cardiac-directed ACVI expression and their transgene-negative littermates (control) underwent coronary ligation, and survival, infarct size, and LV size and function were assessed 1 to 7 days after MI. Mice with increased ACVI expression had increased survival (control 41%, ACVI 74%; P = 0.004). Infarct size and myocardial apoptotic rates were similar in ACVI and control mice; however, ACVI mice had less LV dilation (P < 0.001) and increased ejection fractions (P < 0.03). Three days after MI, studies in isolated perfused hearts showed that basal LV +dP/dt was similar, but graded dobutamine infusion was associated with a more robust LV contractile response in ACVI mice (P < 0.05). Increased LV function was associated with increases in cAMP generation (P = 0.0002), phospholamban phosphorylation (P < 0.04), sarcoplasmic reticulum Ca2+-ATPase (SERCA2a) affinity for calcium (P < 0.015), and reduced AV block (P = 0.04).

Conclusions—In acute MI, increased cardiac ACVI content attenuates adverse LV remodeling, preserves LV contractile function, and reduces mortality. (Circulation. 2006;114:388-396.)

Key Words: gene therapy • receptors, adrenergic, beta • cAMP • coronary disease • signal transduction

Cardiac-directed expression of adenylyl cyclase type VI (ACVI) results in structurally normal hearts with normal basal heart rate and function but supranormal responses to catecholamine stimulation.1 The use of β-adrenergic receptor (βAR)-stimulating agents, which increase intracellular cAMP, may cause sustained myocardial ischemia due to increased myocardial oxygen demand or may induce ventricular arrhythmias. Whether increased cardiac content of ACVI has a deleterious effect on myocardial ischemia is unknown; however, it is reasonable to expect that increased cAMP generation and hence contractile force, with attendant exacerbation of oxygen demand/supply imbalance, would have detrimental consequences in the setting of myocardial infarction (MI) by increasing border zone injury and extending infarct size. Here, we test the hypothesis that increased left ventricular (LV) ACVI content would increase mortality after acute MI. To test this hypothesis, MI was induced by proximal left coronary ligation in transgenic mice with cardiac-directed expression of ACVI and their transgene-negative littermates. We then assessed survival, infarct size, LV size and function, apoptosis rates, cAMP production, calcium handling, and incidence of arrhythmias.

Editorial p 365
Clinical Perspective p 396

Methods

Animals
Animal use and care were in accordance with institutional and National Institutes of Health guidelines. Transgenic mice (C57BL/6) were generated with murine ACVI cDNA under direction of the α-myosin heavy chain promoter to produce cardiac-directed expression of ACVI.1 Male and female ACVI transgenic mice (4 ± 1 months old; n = 108) and their age-matched transgene-negative littermates (control; n = 107) were used. Gene presence was confirmed with genomic DNA purified from tail tips. Mice were housed with free access to food and water and exposed to 12-hour light/dark cycles.

Myocardial Infarction
MI was induced by permanent ligation of the left coronary artery as described previously.2
**Survival Study**

ACV/VI transgenic mice and their transgene-negative littermates underwent acute MI in a randomized, blinded study. This study was designed to determine the 7-day survival of mice after MI; therefore, mice that did not survive the surgical procedure were not included in the analysis. Eighty-four mice (42 ACV/VI, 42 control) underwent surgery for the survival study. Sixteen mice (8 ACV/VI, 8 control) died of surgical complications: 4 mice (2 ACV/VI, 2 control) died before coronary ligation, 7 (4 ACV/VI, 3 control) died after coronary ligation but before extubation, and 5 (2 ACV/VI, 3 control) died immediately after extubation. The remaining 68 mice, consisting of 34 ACV/VI mice (21 males, 13 females) and 34 control mice (22 males, 12 females), were enrolled.

**Infarct Size**

Twenty-four hours after MI, area at risk and infarct size were assessed (n=7 per group) with the Evans blue and 2,3,5-triphenyl-tetrazolium chloride (TTC) staining technique. This time point was selected to avoid differences in hypertrophy of the uninfarcted wall that might result from differences in LV ACV/VI expression. Measurements of the proportion of LV infarcted were therefore not confounded by differences in remodeling between groups that might occur between days 2 and 7. Methods to evaluate infarct size in mice 24 hours after coronary occlusion have been established.

Animals were anesthetized, intubated, and connected to a rodent ventilator. Evans Blue (1%) was injected retrogradely (via catheter) into the carotid artery to delineate the area at risk. Hearts were excised and immersed in 1% agarose and sectioned perpendicular to the long axis into 1-mm slices, which were incubated in 1.0% TTC (Sigma; St. Louis, Mo) for 5 minutes at 37°C. Each slice was weighed and photographed under a microscope. LV area, area at risk, and area of infarction for each slice were determined by planimetry with ImagePro software (Image Processing Solutions, Inc, North Reading, Mass).

**In Vivo Hemodynamics**

LV pressure was measured in intact mice, as described previously, 3 days after MI (8 ACV/VI, 7 control). Mice were anesthetized with ketamine (100 mg/kg IP) and xylazine (2.5 mg/kg IP). After LV pressures were recorded, bilateral vagotomy was performed. LV pressure was recorded at baseline and 45 seconds after bolus injection of isoproterenol (1, 10, and 100 pg/g in 100 μL) at 5-minute intervals. Peak rates of LV pressure development (LV +dP/dt) and relaxation (LV −dP/dt) were determined after acquisition of LV pressure signals at a sampling rate of 3000 per second (Datq DI-400, WinDaq software; Dataq Instruments, Akron, Ohio). Ten sequential beats were averaged for each measurement. Data were recorded and analyzed in a blinded manner.

**Ex Vivo Hemodynamics**

Ex vivo cardiac function in response to βAR stimulation was assessed in isolated perfused hearts, as described previously. 3 days after MI (n=6 per group). Dipyridamole was delivered (1, 3 and 10 μmol/L) at 5-minute intervals as LV pressure was recorded, and LV +dP/dt and −dP/dt were determined. Data were recorded and analyzed in a blinded manner.

**Echocardiography**

Echocardiography was performed with a 16-MHz probe (Sonos 5500, Philips, Bothell, Wash) in mice1 before and 7 days after MI. LV ejection fraction was calculated by the area-length method, which has been validated in rodents and humans.5

**Apoptosis**

Terminal dUTP nick end-labeling (TUNEL) assays were performed on LV samples with the CardioTACS In Situ Apoptosis Detection Kit (R&D Systems, Minneapolis, Minn) as described previously. Seven days after MI, the heart was arrested in diastole, excised, and sliced into 3 sections perpendicular to the long axis. The slices were fixed in 3.7% formaldehyde solution for 24 hours, paraffin embed-
by the Kaplan-Meier method and compared with the log-rank test. EC50 values were estimated and compared with the 3-parameter sigmoidal model. Phospholamban phosphorylation content was compared with a Welch t test. The null hypothesis was rejected if \( P < 0.05 \). Analyses were performed with SPSS for Windows (SPSS, Inc) and EC50 calculations made with GraphPad Prism (GraphPad Software, Inc).

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

Survival Study
Kaplan-Meier analysis revealed increased survival 7 days after MI (the primary end point of the study) in ACVI mice (Figure 1). ACVI mice had a survival rate of 74% compared with 41% for the transgene-negative group (\( n = 34 \) for each group, \( P = 0.004 \)). All animals had anterior-wall MI at necropsy. LV rupture was found in 3 ACVI mice and 3 control mice.

Infarct Size
Figure 2 shows the delineated area at risk and infarct size in hearts stained with Evans Blue and TTC 24 hours after left coronary artery ligation. The area at risk was not different between control and ACVI mice (control 51±6%, ACVI 54±6%; \( n = 7 \) for each group; \( P = 0.46 \)). Infarct size was similar in both groups (control 49±7%; ACVI 51±7%; \( n = 7 \) for each group; \( P = 0.54 \)). The proportion of infarction related to the area at risk was also similar in both groups (control 96±2%, ACVI 96±3%; \( n = 7 \) for each group; \( P = 0.71 \)). Histological examination with TTC staining showed that there was no necrosis in the area of the AV node.

In Vivo Hemodynamics
There were no differences in heart rate, LV +\( \frac{dP}{dt} \), and LV −\( \frac{dP}{dt} \) between the ACVI and control groups before vagotomy (Table 1). After vagotomy, heart rate increased to the same extent in both groups, but LV systolic pressure was higher in ACVI mice (\( P = 0.029 \)). ACVI mice also showed increased LV +\( \frac{dP}{dt} \) (\( P = 0.021 \)) and decreased LV −\( \frac{dP}{dt} \) (\( P = 0.029 \)). These changes persisted when mice were stimulated with isoproterenol (Figure 3). These data indicate that an increase in cardiac ACVI content increases LV contractility and relaxation 3 days after MI.

Ex Vivo Hemodynamics
To provide a means to evaluate LV function isolated from reflex activation, the influence of neurohumoral input, and anesthetic agents, hearts were isolated from ACVI and control mice 3 days after MI. Basal LV systolic pressure was higher in ACVI mice (\( P = 0.037 \)). Basal LV +\( \frac{dP}{dt} \) was similar in both groups (control 2136±567 mm Hg/s, ACVI 2500±446 mm Hg/s; \( n = 6 \) for each group; \( P = 0.42 \)). Graded dobutamine infusion revealed increased LV systolic pressure (Figure 4). Basal heart rate did not differ between groups (\( P = 0.132 \)).

Echocardiography
Table 2 shows echocardiographic findings before and after MI. Before MI, there were no differences in heart rate, LV dimensions, wall thickness, and LV function between ACVI and control mice, as anticipated from our previous report.\(^1\) Seven days after MI, heart rate and posterior wall thickness

---

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Rate (bpm)</th>
<th>LV End-Diastolic Diameter (mm)</th>
<th>LV End-Systolic Diameter (mm)</th>
<th>LV Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACVI</td>
<td>350±10</td>
<td>4.5±0.1</td>
<td>3.8±0.2</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Control</td>
<td>340±15</td>
<td>4.6±0.2</td>
<td>3.7±0.2</td>
<td>0.28±0.03</td>
</tr>
</tbody>
</table>

---

**Figure 2.** A, Transverse sections of LV at the midventricular level 24 hours after MI; 1% Evans Blue and 1% TTC. B, Area at risk was similar in control (CON) and ACVI mice. C, Infarcts were large (50±2%) and were not different between groups. Bars represent mean value; error bars denote 1 SD. \( n = 7 \) for each group.
We found a 17-fold increase in ACVI protein content in LV samples from ACVI mice versus control (Figure 3). We found no group differences in rates of myocardial apoptosis between control and ACVI mice in the border zone 7 days after MI (control differences in rates of myocardial apoptosis between control and ACVI, 704 and ACVI 167 densitometry units; n=8 for each group; P=0.23) or β3AR protein content (control 129±31 and ACVI 167±67 densitometry units; n=8 for each group; P=0.23). Similarly, we found no group difference in Gαs protein content (control 389±24 and ACVI 392±65 densitometry units; n=8 for each group; P=0.72).

**Calcium Uptake**

To elucidate the mechanism by which increased cardiac AC VI protein leads to increased LV contractile function in the setting of acute MI, we assessed LV calcium signaling, a major regulator of contractile function. Western blotting analyses showed that LV SERCA2a protein content did not differ between groups (control 2176±757 and ACVI 1819±642 densitometry units; n=8 for each group; P=0.57). Although total phospholamban was not altered by increased ACVI expression (Figure 7A), Ser16 phosphorylated phospholamban content was increased 2.4-fold 7 days after MI (P<0.04; Figures 7A and 7B). These data suggest a role of ACVI in increasing SR calcium uptake. We then compared ATP-dependent initial SR calcium uptake rate in viable LV homogenates from both groups. The relationship between calcium uptake and calcium concentration was left-shifted in LV samples from ACVI mice (Figure 7C), and SERCA2a affinity for calcium was increased (EC50: control 3.64 μmol/L, ACVI 1.14 μmol/L; n=8 for each group; P=0.0143; Figure 7D).

**Telemetry**

Mean heart rate, measured with ambulatory telemetry monitors in unanesthetized animals, was similar in control and decreased in both groups; however, LV end-diastolic diameter (P<0.001) and LV end-systolic diameter (P<0.001) were smaller in ACVI mice, which indicates reduced chamber dilation. In addition, ACVI mice showed increased LV ejection fractions (P=0.034). These data suggest that cardiac-directed ACVI expression attenuates LV dilation and improves LV contractile function after MI.

**Apopotosis**

A >13-fold increase of TUNEL-positive nuclei was observed in the peri-infarct border zone compared with the noninfarcted remote zone for both groups (Figure 5). There were no differences in rates of myocardial apoptosis between control and ACVI mice in the border zone 7 days after MI (control 6719±1729 and ACVI 6495±2671 positive nuclei per 106 cells; n=7 for each group; P=0.81). The apoptotic rate in the remote zone was also similar in both groups (control 472±151 and ACVI 496±156 positive nuclei per 106 cells; n=7 for each group; P=0.71).

**LV AC VI, Gαs, β1AR, and β2AR Expression**

We found a 17-fold increase in ACVI protein content in LV samples from ACVI mice versus control (P<0.008; Figures 6A and 6B), and AC-stimulated cAMP generation was 4.2-fold higher in viable LV samples from ACVI versus control mice 7 days after MI (P=0.0002; Figure 6C). Cardiac-directed ACVI expression did not affect basal AC activity. We found no group differences in βAR protein content (control 610±92 and ACVI 704±142 densitometry units; n=8 for each group; P=0.23) or β2AR protein content (control 129±31 and ACVI 167±67 densitometry units; n=8 for each group; P=0.23). Similarly, we found no group difference in Gαs protein content (control 389±24 and ACVI 392±65 densitometry units; n=8 for each group; P=0.72).

**Calcium Uptake**

To elucidate the mechanism by which increased cardiac ACVI protein leads to increased LV contractile function in the setting of acute MI, we assessed LV calcium signaling, a major regulator of contractile function. Western blotting analyses showed that LV SERCA2a protein content did not differ between groups (control 2176±757 and ACVI 1819±642 densitometry units; n=8 for each group; P=0.57). Although total phospholamban was not altered by increased ACVI expression (Figure 7A), Ser16 phosphorylated phospholamban content was increased 2.4-fold 7 days after MI (P<0.04; Figures 7A and 7B). These data suggest a role of ACVI in increasing SR calcium uptake. We then compared ATP-dependent initial SR calcium uptake rate in viable LV homogenates from both groups. The relationship between calcium uptake and calcium concentration was left-shifted in LV samples from ACVI mice (Figure 7C), and SERCA2a affinity for calcium was increased (EC50: control 3.64 μmol/L, ACVI 1.14 μmol/L; n=8 for each group; P=0.0143; Figure 7D).

**Telemetry**

Mean heart rate, measured with ambulatory telemetry monitors in unanesthetized animals, was similar in control and decreased in both groups; however, LV end-diastolic diameter (P<0.001) and LV end-systolic diameter (P<0.001) were smaller in ACVI mice, which indicates reduced chamber dilation. In addition, ACVI mice showed increased LV ejection fractions (P=0.034). These data suggest that cardiac-directed ACVI expression attenuates LV dilation and improves LV contractile function after MI.

**Apopotosis**

A >13-fold increase of TUNEL-positive nuclei was observed in the peri-infarct border zone compared with the noninfarcted remote zone for both groups (Figure 5). There were no differences in rates of myocardial apoptosis between control and ACVI mice in the border zone 7 days after MI (control 6719±1729 and ACVI 6495±2671 positive nuclei per 106 cells; n=7 for each group; P=0.81). The apoptotic rate in the remote zone was also similar in both groups (control 472±151 and ACVI 496±156 positive nuclei per 106 cells; n=7 for each group; P=0.71).

**LV AC VI, Gαs, β1AR, and β2AR Expression**

We found a 17-fold increase in ACVI protein content in LV samples from ACVI mice versus control (P<0.008; Figures 6A and 6B), and AC-stimulated cAMP generation was 4.2-fold higher in viable LV samples from ACVI versus control mice 7 days after MI (P=0.0002; Figure 6C). Cardiac-directed ACVI expression did not affect basal AC activity. We found no group differences in βAR protein content (control 610±92 and ACVI 704±142 densitometry units; n=8 for each group; P=0.23) or β3AR protein content (control 129±31 and ACVI 167±67 densitometry units; n=8 for each group; P=0.23). Similarly, we found no group difference in Gαs protein content (control 389±24 and ACVI 392±65 densitometry units; n=8 for each group; P=0.72).

**Calcium Uptake**

To elucidate the mechanism by which increased cardiac ACVI protein leads to increased LV contractile function in the setting of acute MI, we assessed LV calcium signaling, a major regulator of contractile function. Western blotting analyses showed that LV SERCA2a protein content did not differ between groups (control 2176±757 and ACVI 1819±642 densitometry units; n=8 for each group; P=0.57). Although total phospholamban was not altered by increased ACVI expression (Figure 7A), Ser16 phosphorylated phospholamban content was increased 2.4-fold 7 days after MI (P<0.04; Figures 7A and 7B). These data suggest a role of ACVI in increasing SR calcium uptake. We then compared ATP-dependent initial SR calcium uptake rate in viable LV homogenates from both groups. The relationship between calcium uptake and calcium concentration was left-shifted in LV samples from ACVI mice (Figure 7C), and SERCA2a affinity for calcium was increased (EC50: control 3.64 μmol/L, ACVI 1.14 μmol/L; n=8 for each group; P=0.0143; Figure 7D).

**Telemetry**

Mean heart rate, measured with ambulatory telemetry monitors in unanesthetized animals, was similar in control and

### TABLE 1. Hemodynamic Data: In Vivo Study

<table>
<thead>
<tr>
<th></th>
<th>Control (n=7)</th>
<th>ACVI (n=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre vagotomy</td>
<td>Post vagotomy</td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>252±113</td>
<td>240±75</td>
<td>0.999; 0.613; 0.779</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>72±8</td>
<td>74±14</td>
<td>0.955; 0.006; 0.029</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>8±4</td>
<td>11±5</td>
<td>0.232; 0.281; 0.955</td>
</tr>
<tr>
<td>LV +dP/dt, mm Hg/s</td>
<td>3505±994</td>
<td>3222±1121</td>
<td>0.536; 0.002; 0.021</td>
</tr>
<tr>
<td>LV −dP/dt, mm Hg/s</td>
<td>2887±771</td>
<td>2706±836</td>
<td>0.694; 0.006; 0.029</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVSP, LV systolic pressure; and LVEDP, LV end-diastolic pressure. Data are mean±SD. *P values are for group differences for prevagotomy (first value), postvagotomy (second value), and change (third value).

**Figure 3.** Cardiac responsiveness to βAR stimulation in vivo, assessed 3 days after MI. A, Basal and isoproterenol-stimulated (1 pg/g) LV +dP/dt were increased in mice with cardiac-directed ACVI expression. B, Basal and isoproterenol-stimulated (1 and 10 pg/g) LV −dP/dt were decreased in mice with cardiac-directed ACVI expression. C, Heart rates were similar in both groups. ● indicates mean values from 7 control mice; ◊, mean values from 8 ACVI mice; and HR, heart rate. Error bars denote 1 SD. *P<0.05 for comparisons of specific dose.
ACVI mice before MI (control 560±34 bpm, ACVI 579±52 bpm; n=8 for each group; P=0.33), as anticipated from a previous report.11 After coronary occlusion, we observed ST-segment elevation and subsequent formation of abnormal Q waves in each animal (Figure 8). Mean heart rate after MI was comparable between control and ACVI mice (control 492±84 bpm, ACVI 489±110 bpm; n=8 for each group; P=0.88). Total number of premature ventricular complexes during the first 24 hours after MI was not different (control 2000±1714, ACVI 5056±6581; n=8 for each group; P=0.57). The proportion of animals showing nonsustained ventricular tachycardia, a frequent occurrence after MI, was not different between groups (control 50%, ACVI 50%; P=0.99). ACVI mice had a reduced incidence of second- or third-degree AV block during the first 24 hours after MI (control 75%, ACVI 13%; P=0.02). In this subset of animals with telemetry units, 2 of 8 ACVI transgenic mice versus 4 of 8 control mice died by the seventh day after MI; LV rupture was found in 1 ACVI and 1 control mouse. The agonal rhythm consistently was marked bradycardia with progressive high-grade AV block (Figure 8). Sustained ventricular tachycardia or fibrillation did not occur.

Propranolol Study

Table 3 summarizes the results from the secondary study on the effects of propranolol compared with ACVI. Propranolol and ACVI had similar survival advantages (propranolol: 63% survival, n=19; ACVI: 74% survival, n=34; P=0.44) and similar effects on LV ejection fraction (propranolol: 20±6%, n=10; ACVI: 25±6%, n=11; P=0.09). Apoptosis rates were similar in propranolol and ACVI groups in both border and remote zones (Table 3).

Discussion

In acute MI, increased cardiac myocyte ACVI expression is associated with reduced mortality (56% reduction; P=0.004). The unexpected favorable effect on mortality conferred by ACVI expression led us to seek mechanisms for increased survival. We therefore measured infarct size, extent of dysfunction, degree of remodeling, apoptosis rates, cAMP generation, calcium handling, and incidence of arrhythmic events.

We asked whether increased cardiac ACVI content influenced infarct size, because LV contractile function and mortality after MI are closely linked with infarct size. There were no differences in area at risk or infarct size in a subgroup of ACVI and control mice. Infarct size averaged 50% of the LV and was not affected by increased cardiac ACVI expression (Figure 2). Evaluation of LV function was performed 3 days after MI, when survival rates were comparable between the 2 groups. We measured LV function using 2 approaches: in vivo (in anesthetized, ventilated animals) and ex vivo (isolated, perfused, isovolumically contracting hearts). This was done because there are advantages and shortcomings to either approach. The apparent differences in basal LV +dP/dt and LV −dP/dt observed in the “unstimulated” state (no

---

**TABLE 2. Echocardiographic Data**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=11)</th>
<th>ACVI (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before MI</td>
<td>After MI</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>462±40</td>
<td>414±49</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.7±0.2</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.2±0.2</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>AWh, mm</td>
<td>0.68±0.04</td>
<td>0.44±0.05</td>
</tr>
<tr>
<td>PWh, mm</td>
<td>0.65±0.04</td>
<td>0.56±0.09</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>61±5</td>
<td>17±5</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; AWh, anterior-wall thickness; PWh, posterior-wall thickness; and LVEF, LV ejection fraction.

Data are mean±SD.

P values are group differences before MI (first value), after MI (second value), and change (third value).
isoproterenol infused) may not reflect a true basal state, because surgical intervention, anesthesia, and mechanical ventilation are known to increase endogenous catecholamine release, thereby obfuscating assessment of basal heart function.

In the in vivo studies, we found increased LV $\frac{dP}{dt}$ and reduced LV $-\frac{dP}{dt}$ in ACVI mice after vagotomy and after βAR stimulation, which indicates that increased cardiac ACβ1 content increases global LV contractile function and relaxation in the setting of MIs of equivalent size. Vagotomy had similar effects on heart rate in both groups, which suggests that vagal tone was similar. Thus, the differences we saw in LV $\frac{dP}{dt}$ were not likely to be the result of variations in vagal tone. We previously reported that there were no differences in heart rate variability or response to atropine between ACβ1 transgene-positive and -negative mice in studies conducted in conscious ambulatory mice with telemetry,11 which indicates that increased cardiac ACβ1 expression does not alter vagal tone.

To evaluate LV function isolated from reflex activation, neurohumoral input, and anesthesia, we assessed LV contractile function in isolated perfused hearts. These studies showed that basal LV $\frac{dP}{dt}$ was unchanged (Figure 4); however, βAR stimulation was associated with more robust LV systolic pressure development in ACβ1 mice. Increased cardiac reserve would be expected to confer a survival advantage13 and is a likely mechanism for reduced mortality.

Echocardiography was used to assess LV size and function in vivo. There was reduced LV dilation 7 days after MI in ACβ1 mice, which suggests a protective effect of increased cardiac ACβ1 content on adverse LV remodeling after acute MI. Both infarct expansion and dilation of noninfarcted viable regions play a role in the remodeling process that occurs early after MI. Infarct size has a great impact on this deleterious process, but in the present study, infarct sizes were not different between groups. The precise mechanisms...

---

**Figure 5.** A, Photomicrograph showing apoptotic nuclei (dark-stained) in section of LV from the border zone of a control mouse; TUNEL staining, ×400. B, Apoptotic rate in the border zone was similar in control (CON) and ACβ1 mice 7 days after MI. C, Apoptotic rate in the remote zone was decreased compared with the border zone but was not different between groups. Bars represent mean value; error bars denote 1 SD. n = 7 for each group.

---

**Figure 7.** Cardiac-directed ACβ1 expression increased phospholamban (PLN) phosphorylation and calcium uptake by SR. A, Increased phosphorylated PLN content was found in LV samples from ACβ1 mice compared with transgene-negative littermate control (CON) mice; total PLN was unchanged. B, Quantification of immunoblotting showed that phosphorylated PLN content was 2.4-fold higher in LV samples from ACβ1 mice than from control mice (n = 8 for each group); dens indicates densitometry. C, Increased LV ACβ1 was associated with increased SERCA2a calcium sensitivity (n = 8 for each group). D, ACβ1 expression increases SERCA2a affinity for calcium (n = 8 for each group). Data represent mean ± SD (A, B, and C), or SEE (D).
by which increased expression of AC_VI attenuates adverse LV remodeling remain uncertain. Increased function in the non-infarcted viable region may contribute to preservation of global LV function and may decrease activation of the renin-angiotensin-aldosterone system and thereby reduce LV chamber dilation. Reduced LV dilation after MI would be expected to confer a survival advantage and provides a second contributing mechanism for reduced mortality.

Abnormal LV calcium handling is a hallmark of heart failure. Both defective calcium uptake by SERCA2a and defective calcium release through the SR calcium release channel ryanodine receptor 2 (RyR2) occur in clinical and animal models of heart failure. In the present study, we found that AC_VI expression was associated with increased SERCA2a affinity for calcium (Figures 7C and 7D). This improvement of calcium uptake by AC_VI was associated with increased phosphorylation of phospholamban at Ser16; protein contents of phospholamban and SERCA2a were unchanged (Figures 7A and 7B). Expression of a Ser16 pseudophosphorylated mutant of phospholamban in an animal model of cardiomyopathy was reported to increase calcium uptake and attenuate heart failure progression. The present data provide a mechanism by which AC_VI has beneficial effects on LV function and survival after MI.

Because myocardial apoptosis may contribute to the progression of LV remodeling and heart failure, we assessed myocardial apoptosis in the remote and the border zones 7 days after MI. There were no significant differences between groups in apoptotic rates in either region, which indicates that the salutary effects of increased cardiac AC_VI content on LV remodeling after acute MI are not the result of reduced apoptosis. Nonetheless, it is noteworthy that increased cardiac AC_VI expression does not alter myocyte apoptosis, because this result is in contrast to cardiac-directed expression of β1AR or Gz, which is associated with increased myocyte apoptosis and heart failure.

We previously showed that mice with cardiac-directed expression of AC_VI have ambulatory heart rates similar to transgene-negative littermates. In the present study, we found that increased cardiac AC_VI expression, in the setting of acute MI, does not increase mean heart rate or the frequency of ventricular arrhythmias after MI. These data suggest that cardiac AC_VI expression does not increase susceptibility to ventricular arrhythmias, unlike traditional sympathomimetic agents.

TABLE 3. Propranolol Study

<table>
<thead>
<tr>
<th></th>
<th>Control (n)</th>
<th>AC_VI (n)</th>
<th>Propranolol (n)</th>
<th>AC_VI + Propranolol (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Survival</td>
<td>41 (34)</td>
<td>74 (34)</td>
<td>63 (19)*</td>
<td>78 (18)</td>
</tr>
<tr>
<td>Border apoptosis (+nuc per 10^6 cells)</td>
<td>6719±1729 (7)</td>
<td>6495±2671 (7)</td>
<td>6254±1490 (5)</td>
<td>4868±1794 (5)</td>
</tr>
<tr>
<td>Remote apoptosis (+nuc per 10^6 cells)</td>
<td>476±151 (7)</td>
<td>496±151 (7)</td>
<td>448±69 (5)</td>
<td>352±149 (5)</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>17±5 (11)</td>
<td>25±6 (11)</td>
<td>19±6 (10)†</td>
<td>26±8 (11)</td>
</tr>
</tbody>
</table>

+nuc indicates positive (apoptotic) nuclei; LVEF, LV ejection fraction.

Data represent mean±SD. Number in parentheses indicates group size.

*P=0.44 vs AC_VI; †P=0.09 vs AC_VI.
interventions. Non sustained ventricular tachycardia was frequently observed early after MI, but sustained ventricular tachycardia or ventricular fibrillation was not seen. We found that the agonal rhythm consistently was bradycardia with progressive AV block. In clinical settings, MI of the large size that we induced in the present study (50% of LV) would be associated with severe heart failure. Bradycardia is commonly seen as a terminal rhythm in patients with severe heart failure. In murine models of myocardial ischemia/infarction and heart failure, very few instances of ventricular tachycardia or ventricular fibrillation have been documented, and the usual agonal rhythm is bradycardia.

We documented reduced mortality and reduced incidence of second- and third-degree AV block (P=0.02) in mice with cardiac-directed ACVI expression. Recently, using electrophysiological approaches in transgenic mice, we found that cardiac-directed expression of ACVI facilitated AV conduction through a wide range of heart rates. Increased cardiac ACVI expression, by facilitating AV conduction, would be predicted to have a protective effect on fatal bradyarrhythmias associated with MI and is likely to have played a role in the mechanism for reduced mortality that we found in the present study.

ACVI and propranolol had similar salutary effects on mortality and LV remodeling in the setting of acute MI. Those who assume that ACVI gene transfer recapitulates βAR stimulation will think that these results are counterintuitive. However, given their unique roles as signaling molecules—one a βAR antagonist, the other an effector molecule for multiple G protein–coupled receptor pathways—one would not predict equal and opposite effects. Furthermore, βAR stimulation has effects on transcription and expression of key proteins important in cardiac function (eg, βAR, phospholamban, and atrial natriuretic factor) that are directionally opposite to those evoked by ACVI gene transfer.

Clinical Implications
An American College of Cardiology/American Heart Association consensus panel recommends the use of βAR antagonists early in clinical acute MI, and clinical trials indicate that the use of βAR antagonists in this setting reduces mortality. Having established that increased cardiac content of ACVI has an unanticipated favorable effect on survival in acute MI, we performed a secondary study to determine the effects of propranolol (versus ACVI) on mortality and LV function, and apoptosis rates in the present model. Propranolol had favorable effects on survival and LV function that were not statistically different from the effects of ACVI, and like ACVI, propranolol did not alter apoptosis rates at this early time point after MI. The precise mechanism by which propranolol has a favorable effect on LV remodeling in clinical settings in the acute phase of MI is not known precisely, although favorable effects are associated with reduced apoptosis in longer-term studies. On the basis of the present data, we would anticipate that ACVI gene transfer may have a beneficial effect on survival in acute MI in clinical settings. This effect of ACVI does not negate and may even increase the beneficial effects of βAR blockade.

In conclusion, increased cardiac ACVI content reduces mortality in acute MI without affecting infarct size. Three mechanisms contribute to this survival advantage: increased LV contractile responsiveness, reduced LV dilatation, and reduced incidence of high-grade AV block. The molecular underpinning for favorable effects on LV function includes increased LV cAMP-generating capacity and calcium handling.

Acknowledgments
This work was supported by National Institutes of Health grants P01 HL66941 and HL081741 (Dr Hammond), Merit review awards from the Department of Veteran’s Affairs (Drs Roth, Lew, and Hammond), and a fellowship from the Banyu Life Science Foundation International (Dr Takahashi). We thank Matthew Spellman, Jesus Jiménez, and Evelyn Bayna for technical assistance.

Disclosures
None.

References
Clinical trials of cardiac gene transfer of adenylyl cyclase type VI (ACvI) for heart failure may soon be initiated. Increased cardiac ACvI in transgenic mice results in structurally normal hearts with normal basal heart rate and function but supranormal responses to catecholamine stimulation. The use of β-adrenergic receptor (βAR)–stimulating agents, which increase intracellular cAMP, may cause sustained myocardial ischemia due to increased myocardial oxygen demand or may induce ventricular arrhythmias. Whether increased cardiac ACvI has a deleterious effect on myocardial ischemia is unknown; however, increased cAMP generation and contractile force may be detrimental in the setting of myocardial infarction (MI) by increasing border zone injury and extending infarct size. In the present study, MI was induced by proximal left coronary ligation in transgenic mice with cardiac-directed expression of ACvI and their transgene-negative littermates. We then assessed survival, infarct size, LV size and function, apoptosis rates, cAMP production, calcium handling, and incidence of arrhythmias. We found that increased ACvI content reduces mortality in acute MI without affecting infarct size. Three mechanisms contribute to this survival advantage: increased LV contractile responsiveness, reduced LV dilation, and reduced incidence of high-grade AV block. The molecular underpinning for favorable effects on LV function includes increased LV cAMP-generating capacity and calcium handling. On the basis of the present data, we would anticipate that ACvI gene transfer may have a beneficial effect on survival in acute MI in clinical settings. This effect of ACvI does not negate and may even increase the beneficial effects of βAR blockade.
Increased Cardiac Adenylyl Cyclase Expression Is Associated With Increased Survival After Myocardial Infarction
Toshiyuki Takahashi, Tong Tang, N. Chin Lai, David M. Roth, Brian Rebolledo, Miho Saito, Wilbur Y.W. Lew, Paul Clopton and H. Kirk Hammond

Circulation. 2006;114:388-396; originally published online July 24, 2006; doi: 10.1161/CIRCULATIONAHA.106.632513
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/114/5/388

An erratum has been published regarding this article. Please see the attached page for:
/content/114/11/e497.full.pdf

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
In the version of the article “Increased Cardiac Adenylyl Cyclase Expression Is Associated With Increased Survival After Myocardial Infarction” by Takahashi et al that published online before print on July 24, 2006, and appeared in the August 1, 2006, issue of the journal (Circulation. 2006;114:388–396), incorrect graphs were supplied for Figure 2B and 2C. The mistake did not change the results or conclusions of the article. The figure has been corrected in the current online version. The authors regret this error.

DOI: 10.1161/CIRCULATIONAHA.106.178308