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

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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 β 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.

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

ACVI transgenic mice and their transgene-negative littermates under- went 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 ACVI; 42 control) underwent surgery for the survival study. Sixteen mice (8 ACVI; 8 control) died of surgical complications: 4 mice (2 ACVI; 2 control) died before coronary ligation, 7 (4 ACVI; 3 control) died after coronary ligation but before extubation, and 5 (2 ACVI; 3 control) died immediately after extubation. The remaining 68 mice, consisting of 34 ACVI 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 hypotherphy of the uninfarcted wall that might result from differences in LV ACVI expression. Measurements of the proportion of LV infarcted were therefore not con- founded 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% agarse 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 ACVI, 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 (Daq 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 measured in isolated perfused hearts, as described previously, 3 days after MI (n=6 per group). Dobutamine 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 mice 1 before and 7 days after MI. LV ejec- tion fraction was calculated by the area-length method, which has been validated in rodents and humans.

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 com-
pared 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 nec-
ropsy. 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 $+dP/dt$, and LV $-dP/dt$ between the ACVI and control groups before vagot-
yomy (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 $+dP/dt$ ($P = 0.021$) and decreased LV $-dP/dt$ 
($P = 0.029$). These changes persisted when mice were stimu-
lated 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 $+dP/dt$ 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

Figure 1. Kaplan-Meier curve showing survival after MI in ACVI 
mice (n = 34) and control (CON) mice (n = 34). Mortality at 7 days 
was significantly reduced in ACVI mice ($P = 0.004$).

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.
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 ACVI, Gos, 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 β1AR 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

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**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>Prevagotomy</td>
<td>Postvagotomy</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).
AC_v1 mice before MI (control 560±34 bpm, AC_v1 579±52 bpm; n=8 for each group; P=0.33), as anticipated from a previous report. 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 AC_v1 mice (control 492±84 bpm, AC_v1 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, AC_v1 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%, AC_v1 50%; P=0.99). AC_v1 mice had a reduced incidence of second- or third-degree AV block during the first 24 hours after MI (control 75%, AC_v1 13%; P=0.02). In this subset of animals with telemetry units, 2 of 8 AC_v1 transgenic mice versus 4 of 8 control mice died by the seventh day after MI; LV rupture was found in 1 AC_v1 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 AC_v1. Propranolol and AC_v1 had similar survival advantages (propranolol: 63% survival, n=19; AC_v1: 74% survival, n=34; P=0.44) and similar effects on LV ejection fraction (propranolol: 20±6%, n=10; AC_v1: 25±6%, n=11; P=0.09). Apoptosis rates were similar in propranolol and AC_v1 groups in both border and remote zones (Table 3).

**Discussion**

In acute MI, increased cardiac myocyte AC_v1 expression is associated with reduced mortality (56% reduction; P=0.004). The unexpected favorable effect on mortality conferred by AC_v1 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 AC_v1 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 AC_v1 and control mice. Infarct size averaged 50% of the LV and was not affected by increased cardiac AC_v1 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>Control (n=11)</th>
<th>AC_v1 (n=11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before MI</td>
<td>After MI</td>
<td>Before 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>AWth, mm</td>
<td>0.68±0.04</td>
<td>0.44±0.05</td>
</tr>
<tr>
<td>PWth, 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; AWth, anterior-wall thickness; PWth, 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 $+dP/dt$ and reduced LV $-dP/dt$ in ACV$_{\mu}$ mice after vagotomy and after βAR stimulation, which indicates that increased cardiac ACV$_{\mu}$ 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 $dP/dt$ 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 ACV$_{\mu}$ transgene-positive and -negative mice in studies conducted in conscious ambulatory mice with telemetry, which indicates that increased cardiac ACV$_{\mu}$ 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 $+dP/dt$ was unchanged (Figure 4); however, βAR stimulation was associated with more robust LV systolic pressure development in ACV$_{\mu}$ mice. Increased cardiac reserve would be expected to confer a survival advantage 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 ACV$_{\mu}$ mice, which suggests a protective effect of increased cardiac ACV$_{\mu}$ 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...
by which increased expression of ACVI 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 advantage14 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.9,15,16 In the present study, we
found that ACVI expression was associated with increased
SERCA2a affinity for calcium (Figures 7C and 7D). This
improvement of calcium uptake by ACVI was associated with
increased phosphorylation of phospholamban at Ser16; pro-
tein contents of phospholamban and SERCA2a were un-
changed (Figures 7A and 7B). Expression of a Ser16 pseu-
dophosphorylated mutant of phospholamban in an animal
model of cardiomyopathy was reported to increase calcium
uptake and attenuate heart failure progression.17 The present
data provide a mechanism by which ACVI has beneficial
effects on LV function and survival after MI.

Because myocardial apoptosis may contribute to the pro-
gression 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 ACVI content on LV
remodeling after acute MI are not the result of reduced
apoptosis. Nonetheless, it is noteworthy that increased car-
diac ACVI expression does not alter myocyte apoptosis,
because this result is in contrast to cardiac-directed expres-
sion of β1AR or Gαs, which is associated with increased
myocyte apoptosis and heart failure.12,18,19

We previously showed that mice with cardiac-directed
expression of ACVI have ambulatory heart rates similar to
transgene-negative littermates.11 In the present study, we
found that increased cardiac ACVI 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 ACVI expression does not increase susceptibility to
ventricular arrhythmias, unlike traditional sympathomimetic

**Figure 8.** Representative ECGs obtained from a control mouse. A, Normal sinus rhythm at baseline (heart rate 480 bpm). B,
ST-segment elevation after acute MI (heart rate 540 bpm). C, Ventricular tachycardia that continued for 15 seconds (heart rate 1080
bpm). D and E, Agonal rhythm consistently was high-grade AV block with ventricular escape rhythm (heart rate 120 bpm) followed by
asystole.

**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 10⁶ 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 10⁶ 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.20 In murine models of myocardial ischemia/infarction and in heart failure, very few instances of ventricular tachycardia or ventricular fibrillation have been documented, and the usual agonal rhythm is bradycardia.21

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.22 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.23

Clinical Implications

An American College of Cardiology/American Heart Association consensus panel recommends the use of βAR antagonists early in clinical acute MI,24 and clinical trials indicate that the use of βAR antagonists in this setting reduces mortality.25 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, LV function, and 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.26 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 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.

Acknowledgments

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Disclosures

None.

References

Clinical trials of cardiac gene transfer of adenylyl cyclase type VI (ACV6) for heart failure may soon be initiated. Increased cardiac ACV6 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 (BAR)–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 ACV6 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 ACV6 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 ACV6 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 ACV6 gene transfer may have a beneficial effect on survival in acute MI in clinical settings. This effect of ACV6 does not negate and may even increase the beneficial effects of BAR blockade.
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Toshiyuki Takahashi, Tong Tang, N. Chin Lai, David M. Roth, Brian Rebolledo, Miho Saito, Wilbur Y.W. Lew, Paul Clopton and H. Kirk Hammond

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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.

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