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Activation of Cardiac Adenylyl Cyclase Expression Increases Function of the Failing Ischemic Heart in Mice

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Abstract

Objectives—This study sought to evaluate whether increased left ventricular (LV) adenylyl cyclase VI (AC_{VI}) expression, at a time when severe congestive heart failure (CHF) was present, would increase function of the actively failing heart.

Background—Increased LV AC_{VI} content markedly reduces mortality and increases LV function after acute myocardial infarction (MI) in mice. However, the effects of increased cardiac AC_{VI} content in the setting of severe heart failure caused by ischemic cardiomyopathy are unknown.

Methods—Mice with cardiac-directed and regulated expression of AC_{VI} underwent coronary artery ligation to induce severe CHF 5 weeks later. AC_{VI} expression was then activated in 1 group (AC-On) but not the other (AC-Off). Multiple measures of LV systolic and diastolic function were obtained 5 weeks later, and LV samples were assessed for alterations in calcium and beta-adrenergic receptor signaling, apoptosis, and cardiac troponin I phosphorylation.

Results—The LV systolic and diastolic function was increased 5 weeks after activation of AC_{VI} expression. Improved LV function was associated with normalization of cardiac troponin I phosphorylation and reduced apoptosis.

Conclusions—Activation of cardiac AC_{VI} expression in mice with ischemic cardiomyopathy and severe CHF improves function of the failing heart.

Increased adenylyl cyclase VI (AC_{VI}) content reduces mortality and increases left ventricular (LV) function after acute myocardial infarction (MI) (1), and also has beneficial effects in congestive heart failure (CHF) (2–4). Cardiac gene transfer of AC_{VI} attenuates the decline of cardiac function in pacing-induced CHF (2). Increased LV AC_{VI} content prevents CHF from occurring in a genetic model of cardiomyopathy using a crossbreeding strategy (3,4). Other genes have been shown to slow the decline of LV function (5), or in genetic models, to prevent heart failure from occurring (6–8). However, studies that have documented increased function of an actively failing heart by increased expression of a putatively therapeutic gene are remarkably rare (9). Demonstration that a candidate gene's expression leads to increased function of the failing heart in a relevant animal model is an

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essential criterion for identifying potentially useful genes for the treatment of clinical CHF. In the present study we ask whether activation of AC_{VI} expression—in the presence of severe CHF caused by MI—would have beneficial effects.

We used a transgenic line that provides cardiac-specific expression of AC_{VI} under tet-regulation (10). This enabled rapid activation of cardiac transgene AC_{VI} expression at any desired point in time. Left coronary artery occlusion was used to induce severe CHF in mice (11), providing a suitable model of clinical ischemic cardiomyopathy, a common etiology for clinical CHF. When evidence of severe CHF was present (5 weeks after MI), we activated transgene cardiac AC_{VI} expression in 1 group; transgene suppression was maintained in the other group. Five weeks after activation of AC_{VI} expression, physiological and molecular studies were conducted and the 2 groups were compared (Fig. 1). Our hypothesis was that increased cardiac expression of AC_{VI}, in the setting of severe CHF, would be associated with increased function of the failing heart. An additional goal was to determine mechanisms for differences in LV function.

Methods

Animals

Animal use and care were in accordance with institutional and National Institutes of Health guidelines.

Transgenic mice with cardiac-directed and regulated (tet-off) expression of AC_{VI} (congenic C57BL/6 background) were generated by our laboratory and previously described in detail (10). Suppression is complete until doxycycline is removed from the water supply (10).

A total of 234 mice were used in the present studies: 174 for the primary comparison and 60 for additional controls (see later text). Of the 174 mice for the AC therapy comparison, 50 mice survived infarction and met the entry criterion: fractional area change (FAC) <30% (normal is >50%). Tetracycline suppression was continued in 1 subgroup (AC-Off), but removed in the other (AC-On) (10). To test the influence of doxycycline on LV size and function, 52 nontransgenic C57BL/6 mice were infarcted. Doxycycline was given in the water supply (as in the previous text) from 10 days before until 5 weeks after MI. For the doxycycline group, 7 met entry criterion; for the no doxycycline group, 11 met entry criterion. We also used 8 nontransgenic C57BL/6 mice as normal control subjects (no MI, no doxycycline treatment).

Heart failure model

We used coronary occlusion to induce large anterior wall MI and CHF as described in detail previously (11). Because MI size deliberately was large, this model is associated with a high initial mortality (>30%); 50% of surviving mice have severe CHF, as has been reported previously (11). The primary end point of the study was LV function 5 weeks after initiation of cardiac-directed AC_{VI} expression in the failing heart (Fig. 1). Group assignment occurred 5 weeks before enrollment. Because we could not predict the number in each group that would meet enrollment criteria 5 weeks later, group sizes were not identical. At enrollment, groups were randomly assigned to continue on doxycycline (AC-Off) or to have it withdrawn (AC-On). Data were acquired and analyzed without knowledge of group identity.

Echocardiography

Echocardiography was performed as previously described (1,2).

In vivo physiology

Mice were anesthetized with sodium pentobarbital (100 mg/kg intraperitoneally), and a 1.4-F conductance-micromanometer catheter (SPR 716, Millar Instruments, Houston, Texas) was inserted via the right carotid artery across the aortic valve and into the LV chamber. After LV pressures were recorded, bilateral vagotomy was performed to minimize confounding effects of reflex activation. End-systolic pressure (ESP) and LV pressure development (+dP/dt) and relaxation (−dP/dt) were obtained. Inferior vena cava occlusion was performed to reduce LV volume, and end-systolic pressure–volume relationship (ESPVR) was obtained. Stroke volume was determined by subtracting the LV end-systolic from the LV end-diastolic volume, measured by conductance catheter; stroke volume × heart rate provided cardiac output.

Calcium uptake

The initial rate of adenosine triphosphate-dependent sarcoplasmic reticulum (SR) calcium uptake in viable LV samples was measured as previously described (12).

LV cyclic adenosine monophosphate (cAMP) generation

Cyclic adenosine monophosphate production in viable LV samples was measured as previously described (1).

Protein kinase and phosphatase activity

Akt was immunoprecipitated from 500-μg LV samples and assayed (Akt Kinase Assay Kit, Cell Signaling Technology, Danvers, Massachusetts). Protein kinase A (PKA) activity (cAMP-dependent) (12) and activities of protein phosphatases 1 (PP1) and 2A (PP2A) were measured.

Western blotting

Viable LV samples were homogenized and underwent Western blotting as described previously (12). Antibodies to cTnI and phospho-Ser23/24-cTnI were obtained from Cell Signaling Technology (Danvers, Massachusetts).

Matrix metalloproteinase (MMP) expression

Quantitative real-time reverse-transcriptase polymerase chain reaction was conducted to compare MMP-2 and -8 messenger ribonucleic acid (mRNA) contents. Primer pairs were MMP2: forward 5′-GAGTTGCAACCTCTTTGTGC-3′ and reverse 5′-CAGGTGTGTAACCAATGATCC-3′; MMP8: forward 5′-CCCAGCACCTATTCACTACC-3′ and reverse 5′-CTGTTCTCAGCTGAGGATGC-3′. Specificity of each reverse-transcriptase polymerase chain reaction was checked by its dissociation curve and agarose gel electrophoresis.

Apoptosis

Terminal 2′-deoxyuridine 5′-triphosphate nick end-labeling (TUNEL) assays were performed as described previously (1).

Caspase 3/7 activity

Caspase 3/7 activity in LV homogenates was measured using the Caspase-Glo 3/7 Assay (Promega, Madison, Wisconsin).

Necropsy and histology

Body and LV weights were recorded. Transmural LV samples from regions bordering and remote from the infarction were formalin-fixed; hematoxylin-and-eosin and Masson trichrome stainings were used to assess inflammation and fibrosis. A pathologist scored LV samples for the presence of inflammation and fibrosis (blinded analysis).

Statistical analysis

Results are shown as mean ± SE. Group comparisons were made using the Student *t* test (2-tailed) or 2-way analysis of variance with Bonferroni *t* testing. For histological studies, the degree of inflammation and fibrosis were rated (0, none; 1, mild; 2, moderate; 3, severe), and the Mann-Whitney *U* test was used to test for difference between groups. The null hypothesis was rejected when $p < 0.05$. Analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, California).

Results

Animal model

In previous studies using the same MI procedures, we have shown that infarct size averages 49 ± 3% of the total LV (11), comprising most of the LV free wall. Such large infarctions result in high mortality and severe CHF in survivors. In the present study, 29% of the animals survived infarction, developed CHF, and entered into the study, a typical outcome with this model. Data were not adversely affected by this because we had strict criteria for enrollment into the study: surviving animals had to have substantial LV dysfunction. There were no group differences in body weight (AC-Off: 27.2 ± 0.7 g, n = 11; AC-On: 28.8 ± 1.5 g, n = 8; p = 0.30), LV weight (AC-Off: 138 ± 6 mg, n = 11; AC-On: 130 ± 4 mg, n = 8; p = 0.37) or LV/body weight ratio (AC-Off: 5.1 ± 0.2 mg/g, n = 11; AC-On: 4.6 ± 0.3 mg/g, n = 8; p = 0.17). However, there was LV hypertrophy in both groups compared with uninfarcted mouse hearts where the LV/body weight ratio is 3.5 ± 0.1 mg/g (11). We previously found that the lung weight/tibial length ratio is increased in this model of CHF (p = 0.037) (11). Activation of transgene provided a 16-fold increase in LV AC_{VI} ($p < 0.0001$) (Fig. 2), confirming the efficiency of the tet-regulated transgene (10).

Echocardiography

Basal heart rates were similar in both groups both before and after treatment (Table 1). The LV end-diastolic and end-systolic dimensions were increased 5 weeks after MI, and activation of transgene AC_{VI} expression had no effect on LV dimensions, which did not change from 5 weeks through 10 weeks after MI and CHF. Thicknesses of the posterior and septal walls were reduced by MI, but did not change during treatment. However, there were increases in the LV fractional area change (p = 0.0001) and ejection fraction (EF) (p = 0.0001) (Table 1). Pre-treatment EF was the same in both groups: 24%. Post-treatment EF in the AC-On group was 6 percentage units greater than that in the AC-Off group, a 27% relative increase (AC-Off: 22 ± 2%, n = 15; AC-On: 28 ± 2%, n = 19; p = 0.02).

LV contractile function

To assess the effect of AC_{VI} transgene expression on cardiac contractile function, we measured LV pressure and volume to assess ESPVR. Activation of AC_{VI} expression was associated with increases in basal LV +dP/dt (p = 0.013) and cardiac output (70% increase; p = 0.029) (Table 2). The ESPVR, a relatively load-independent measure of contractile function, was increased 4-fold (p = 0.014) by activation of AC_{VI} expression (Table 2, Fig. 3). Because of difficulties with catheter engagement, sample sizes differ in Table 2 compared with Table 1.

LV diastolic function

Activation of AC_{VI} expression had favorable effects on LV end-diastolic pressure ($p = 0.039$), basal LV $-dP/dt$ ($p = 0.046$), and tau ($p = 0.0008$), indicating increased cardiac relaxation (Table 2).

Effects of doxycycline

We included 2 additional control groups: 1) doxycycline: these nontransgenic C57BL/6 mice received doxycycline continuously from 10 days before until 5 weeks after MI, a protocol similar to the AC-On group; and 2) no doxycycline: these nontransgenic C57BL/6 mice were included as a control for the doxycycline group. There were no differences in any measure of LV size or function 10 weeks after MI in these 2 groups (Table 3), and these values closely matched those in the AC-Off group (unpaired Student *t* test) (Table 1).

LV Ca^{2+} handling

Activation of AC_{VI} expression did not alter the maximal velocity of Ca^{2+} uptake (AC-Off: 268 ± 31 nmol/mg/min, n = 9; AC-On: 292 ± 8 nmol/mg/min, n = 7; p = 0.47) or the affinity constant of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) for Ca^{2+} (K_{Ca}) (AC-Off: 1.32 ± 0.51 μM, n = 9; AC-On: 1.25 ± 0.50 μM, n = 7; p = 0.87), although the values for K_{Ca} were increased compared with normal mice (12). There were no group differences in the following proteins: SERCA2a, Ser16 phosphorylated phospholamban (PLN), Thr17 phosphorylated PLN, calsequestrin, RyR2, and Ser2809 phosphorylated RyR2.

LV cAMP generation

Activation of transgene AC_{VI} was associated with a 2-fold increase in NKH477-stimulated cAMP production in LV membranes ($p = 0.0001$), basal cAMP was unchanged, and PKA activity was increased (Fig. 4). The PKA catalytic subunit expression was unchanged (data not shown). Stimulation by NKH477 of LV samples from age-matched normal mice show 121 ± 10 pmol/mg/min cAMP generation (n = 8), indicating that LV cAMP-generating capacity is reduced by 28% in untreated CHF ($p = 0.02$) and increased to 45% above normal levels after activation of AC_{VI} expression ($p = 0.006$). There were no group differences in protein contents of β_1AR, β_2AR, or Gαs.

Phosphorylation of cTnI

Activation of cardiac expression of AC_{VI} in the setting of CHF was associated with a 2.7-fold increase in cTnI phosphorylation at Ser23/24 ($p = 0.01$) (Fig. 5). The LV samples from normal mice have levels of cTnI phosphorylation at Ser23/24 of 361 ± 34 densitometric unit (du) (n = 8), indicating that severe CHF is associated with a 70% reduction in cTnI phosphorylation at Ser23/24 ($p < 0.0001$). Activation of AC_{VI} expression in failing hearts restored cTnI phosphorylation at Ser23/24 to normal ($p = 0.33$). The LV samples showed no differences in protein phosphatase PP1 or PP2A content (PP1: AC-Off: 2,516 ± 62 du, n = 8; AC-On: 2,431 ± 83 du, n = 8; p = 0.43; PP2A: AC-Off: 770 ± 55 du, n = 8; AC-On: 728 ± 123 du, n = 8; p = 0.76) or activity (PP1: AC-Off: 1.01 ± 0.12 nmol/mg/min, n = 8; AC-On: 1.24 ± 0.08 nmol/mg/min, n = 8; p = 0.43; PP2A: AC-Off: 1.04 ± 0.06 nmol/mg/min, n = 8; AC-On: 1.22 ± 0.10 nmol/mg/min, n = 8; p = 0.12).

MMP expression

There were no group differences in LV expression of MMP2 mRNA (AC-Off: 100 ± 15%, n = 5; AC-On: 110 ± 17%, n = 7; p = 0.68) or MMP8 mRNA (AC-Off: 100 ± 31%, n = 5; AC-On: 85 ± 52%, n = 7; p = 0.83).

Apoptosis

TUNEL staining was used to quantify apoptosis in the border and remote zones 10 weeks after MI. The LV samples from AC-Off mice showed a 7.3-fold increase in apoptosis in the border zone and a 3.9-fold increase in the remote zone compared with baseline LV apoptosis (1). Activation of AC_{VI} expression was associated with a 59% reduction of apoptosis in the border zone and a 46% reduction in the remote zone ($p = 0.024$) (Fig. 6). There was no group difference in LV Bcl-2 protein content (AC-Off: 205 ± 26 du, n = 8; AC-On: 201 ± 53 du, n = 8; $p = 0.94$) or caspase 3/7 activity (AC-Off: 828 ± 92 relative light units (RLU)/μg, n = 9; AC-On: 654 ± 71 RLU/μg, n = 7; $p = 0.18$), and LV Akt activity was reduced by activation of AC_{VI} expression (AC-Off: 1,199 ± 93 du, n = 8; AC-On: 722 ± 74 du, n = 8; $p = 0.001$).

Histology

There was evidence of mild to moderate inflammation and fibrosis, which were more apparent in areas adjacent to the infarction than in the remote regions. There were no group differences in these findings.

Discussion

A key finding of the study is improved systolic and diastolic LV function 5 weeks after activation of cardiac AC_{VI} expression in the setting of severe CHF because of ischemic cardiomyopathy. Animals in the AC-Off group showed a decline in LV ejection fraction between weeks 5 and 10. In contrast, after AC_{VI} expression was activated, LV ejection fraction increased during the same interval, substantiating a true treatment effect. LV +dP/dt, a measure of LV systolic function, was increased by activation of cardiac AC_{VI} expression. In addition, the slope of the ESPVR—perhaps the best measurement of contractile function of the intact heart—was increased 4-fold. This confirms a marked AC_{VI} effect on contractile function of the failing heart, and likely explains the increase in cardiac output. Furthermore, 3 measures of diastolic function—LV end-diastolic pressure, LV −dP/dt, and tau, were all decreased by activation of cardiac AC_{VI} expression, documenting improved diastolic function.

About 70% of patients with severe CHF have previous MI as the cause, thus coronary occlusion with subsequent LV chamber dilation and CHF provided a suitable model. Our methods result in infarction of 49 ± 3% of the LV and septum, with a transmural scar comprising the majority of the LV free wall (11). Pre-treatment LV ejection fraction was 24% in both groups, the result of large MI. Our model was relevant to clinical CHF and provided a substantial challenge for therapeutic intervention. An additional critical feature of this study is that transgene expression was activated only after severe CHF was present, thus providing a stringent test for efficacy. This was achieved using a cardiac-directed and tet-regulated transgene. We previously have shown that transgene expression is undetectable during doxycycline suppression (10). Once severe CHF was documented, 1 group continued to receive doxycycline and thus transgene suppression (AC-Off), whereas the other group no longer received doxycycline, which activated cardiac AC_{VI} expression (AC-On). Indeed, we found a 16-fold increase in AC_{VI} protein in LV samples from the AC-On versus AC-Off animals, documenting substantial expression in response to transgene activation.

Tetracyclines, which may attenuate MMP expression and activity, can influence LV remodeling when administered in the first few days after MI (13). Therefore, it was important that all animals received identical treatment during the healing phase of MI. We maintained this inhibition similarly in animals before and 5 weeks after MI. This ensured that scar development, adverse LV remodeling, and degree of heart failure were similar

between groups. The LV samples from the 2 groups showed no differences in MMP2 or MMP8 expression, suggesting that doxycycline treatment, at the dose required for suppression of AC_{VI} expression, did not influence MMP in this model. This was confirmed in additional nontransgenic control animals, which showed similar severe abnormalities in LV size and function 10 weeks after MI with or without doxycycline treatment (Table 3).

We also sought specific mechanisms by which increased expression of AC_{VI} improved LV systolic and diastolic function. We found no change in several signaling proteins that can influence cardiac function—β_1AR, β_2AR, Gαs, SERCA2a, calsequestrin, PLN (and its phosphorylation), and RyR2 (and its phosphorylation) were similar in both groups. Furthermore, no group differences in SR calcium uptake were observed. In 2 previous reports, increased cardiac AC_{VI} content was associated with improved SR calcium uptake (1,12). However, 1 of these studies was conducted in the acute phase of MI (1), and the other used a crossbreeding strategy in which heart failure was prevented rather than treated (12). Our data indicate that increased cardiac expression of AC_{VI} in this setting does not affect LV SR calcium uptake.

We found that cAMP-generating capacity, which is substantially decreased in the failing heart, was returned to normal levels by increased AC_{VI} expression. These data suggest that improved cardiac function was attributable, at least in part, to increased cardiac AC_{VI} expression and associated increases in cAMP-generating capacity. It is important to point out, however, that increased cardiac expression of other βAR signaling elements that increase cAMP (βAR, Gs) have disastrous effects in both normal and failing hearts (6,14,15), so the favorable outcome with AC_{VI} expression is unique, and likely reflects effects of AC_{VI} not solely attributable to cAMP amplification.

Increases in LV contractile function in the absence of alterations in calcium handling suggested that distal changes, perhaps in contractile proteins, were evoked by activation of AC_{VI} expression. We focused on cTnI phosphorylation because, in human failing hearts, dephosphorylation is specific to cTnI, and is thought not to occur among other contractile proteins such as cardiac troponin T, myosin light chain, or myosin binding protein C (16). Activation of cardiac AC_{VI} expression in the setting of CHF was associated 5 weeks later with a 2.7-fold increase in cTnI phosphorylation at Ser23/24. Such an increase would be anticipated to have a positive effect on cardiac function. Transgenic mice with pseudophosphorylated cTnI at Ser23/24 have increased LV contractility (17). Furthermore, phosphorylation of cTnI at Ser23/24 increases the off-rate for Ca^{2+} exchange with cTnC (18), which increases relaxation rate (19). Phosphorylation of cTnI regulates maximal tension development, crossbridge kinetics, and systolic power production in skinned myocardial fiber bundles (19). Reduced phosphorylation of cTnI at Ser23/24 is associated with marked abnormalities in diastolic and systolic function in failing human hearts, and may account for reduced LV contractile function even when calcium handling is relatively normal (16,20).

Untreated heart failure (AC-Off) was associated with a 70% reduction in cTnI phosphorylation at Ser23/24 compared with normal murine LV, and activation of AC_{VI} restored cTnI phosphorylation to normal levels. We found increased phosphorylation of cTnI but not PLN or RyR2—even though activation of AC_{VI} expression increased cAMP-generating capacity and PKA activity. Phosphorylation of cTnI but not PLN has previously been reported in the setting of increased cardiac cAMP content (21). Selective phosphorylation of cTnI cannot easily be explained by AC_{VI} activation of PKA, and indicates a novel association between cardiac AC_{VI} content and cTnI that may reflect differences in compartmentation or association with A-kinase anchoring protein (22). How increased AC_{VI} expression enables selective phosphorylation of cTnI is a focus of ongoing

studies in our laboratory. We have found no previous examples in which a treatment for heart failure increased cTnI phosphorylation. Phosphorylation of cTnI alone, in the absence of alterations in calcium handling, seems to be sufficient for substantial improvement in function of the failing heart (16).

Increased apoptosis in both border (7.3-fold increase) and remote (3.9-fold increase) zones were found in failing hearts 10 weeks after MI (AC-Off). Increased AC_{VI} expression was associated with a 46% to 59% reduction in apoptosis rates ($p = 0.024$). These results are in contrast to cardiac-directed expression of β_1AR or Gαs, which are associated with increased cardiac apoptosis (14,23). A 50% reduction in apoptosis would be anticipated to preserve viable LV mass over time, and thus have implications in longer-term studies and in clinical settings.

LV Bcl-2 protein content and caspase 3/7 activities were not altered by AC_{VI} expression. However, increased LV Akt activity, seen in untreated heart failure (AC-Off), was reduced by activation of AC_{VI} expression. Although expression of constitutively active Akt1 seems to be protective, prolonged Akt activation is associated with contractile dysfunction and CHF (24). The precise molecular mechanism for reduced apoptosis conferred by activation of AC_{VI} expression in CHF will require additional studies in isolated cardiac myocytes.

Despite striking improvement in LV systolic and diastolic function, we did not find changes in LV dimensions 5 weeks after treatment. Increased AC_{VI} expression attenuates adverse remodeling in acute MI (1). However, all animals in the current study had the same low level of AC_{VI} in the heart 5 weeks after large MI, and extensive adverse LV remodeling occurred before treatment was initiated. Sustained AC_{VI} expression in genetic cardiomyopathy prevents deleterious LV remodeling and increases survival (4), and intracoronary delivery of an adenovirus vector encoding AC_{VI} reduces LV enlargement in pacing-induced CHF (2). Whether longer or earlier AC_{VI} expression also will diminish adverse LV remodeling in this model, as it has in other models (1,2,4), is currently being addressed. Pleger et al. (9), using S100A1 gene transfer in MI-induced CHF in rats, found attenuation of LV dilation, unlike our study. Differences on LV remodeling may be caused by smaller MIs in their study. We infarcted 49% of the LV (pre-treatment EF 24%); Pleger et al. (9) infarcted 25% of LV (pre-treatment EF 40%). Their study confirms that gene transfer strategies can effectively treat CHF in pre-clinical models.

Study limitations

The current study was designed to determine whether activation of cardiac AC_{VI} expression could increase function of the failing heart, but was not adequately powered to determine whether mortality was reduced by the intervention. The death rates were similar between groups during the 5-week treatment phase in the current study (AC-Off: 2 deaths; AC-On: 1 death). However, we have shown a mortality advantage of AC_{VI} in acute MI (1) and in murine genetic cardiomyopathy (4), an effect associated with favorable electrical remodeling conferred by AC_{VI} expression (25). The present study shows that global cardiac expression of AC_{VI} has beneficial effects on the failing heart. It remains to be seen whether exogenous gene transfer of AC_{VI} can achieve similar results. However, we recently showed a reduced rate of decline in LV function in pacing-induced CHF after intracoronary delivery of an adenovirus encoding AC_{VI} (2).

Clinical implications

Increased cardiac AC_{VI} expression has favorable effects on function of the failing heart. In previous studies, we have shown that increased cardiac AC_{VI} also has favorable effects on

survival in acute MI (1) and on electrical remodeling in CHF (25). These data provide a rationale for increasing expression of cardiac AC_{VI} in clinical CHF.

Conclusions

In conclusion, our data show that, in an animal model of ischemic cardiomyopathy, increased cardiac expression of AC_{VI} improves function of the failing heart and increases phosphorylation of cTnI.

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Abbreviations and Acronyms

AC_{VI}	adenylyl cyclase VI
cAMP	cyclic adenosine monophosphate
CHF	congestive heart failure
+dP/dt	pressure development
EF	ejection fraction
ESP	end-systolic pressure
ESPVR	end-systolic pressure–volume relationship
FAC	fractional area change
LV	left ventricular
MI	myocardial infarction
MMP	matrix metalloproteinase
PKA	protein kinase A
PP1	protein phosphatases 1
PP2A	protein phosphatases 2A
RLU	relative light units
SR	sarcoplasmic reticulum

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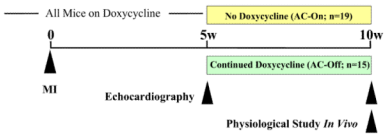

Figure 1. Experimental Design

See the text for a full description.

AC = adenylyl cyclase; MI = myocardial infarction.

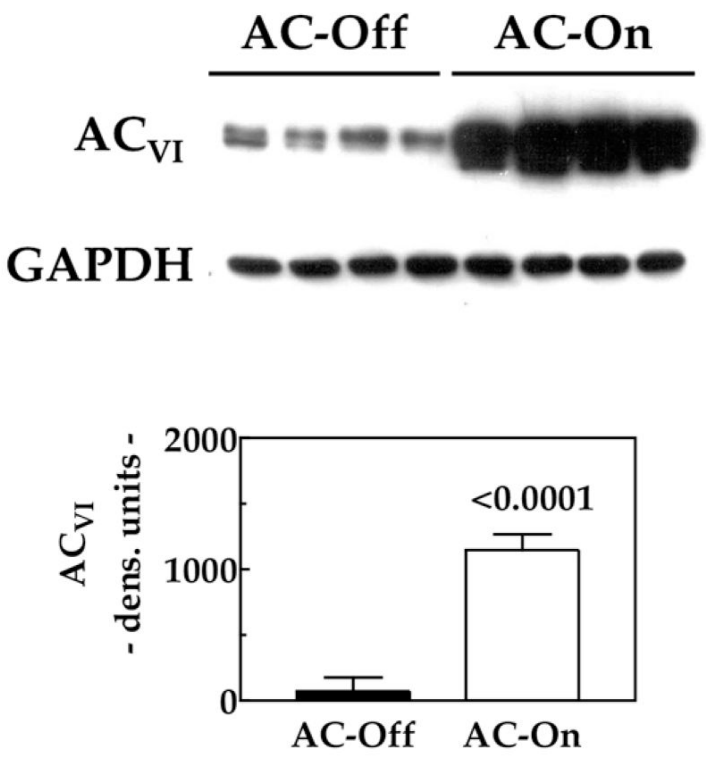

Figure 2. LV AC$_{VI}$ Content

The Western blot shows a marked increase in AC$_{VI}$ protein in LV samples from mice 5 weeks after activation of AC$_{VI}$ transgene expression (n = 8 for both groups). The **graph** summarizes data from Western blotting. **Bars** = mean values; **error bars** = 1 SE; **number above bars** = probability value (Student unpaired *t* test, 2-tailed). AC$_{VI}$ = adenylyl cyclase VI; LV= left ventricular.

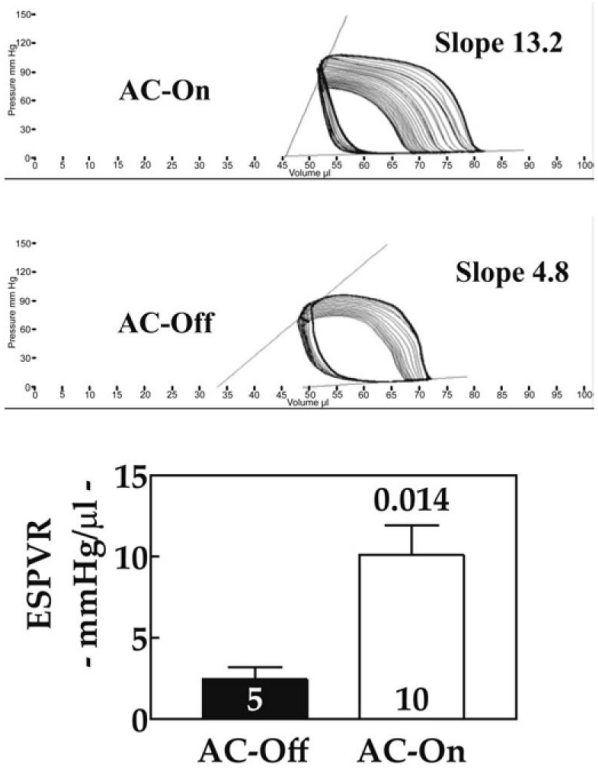

Figure 3. Contractile Function

The ESPVR was measured in intact mice 5 weeks after activation of AC_{VI} expression in animals with severe CHF. Increased cardiac AC_{VI} expression was associated with substantial increases in LV contractility as reflected in increased slope of the ESPVR. Cardiac loops, generated by altering loading conditions of the LV, are shown with the slope of the end-systolic pressure-volume point depicted. The **graph** below summarizes data from all animals. Numbers in bars denote animals studied. **Bars** = mean values; **error bars** = 1 SE; **number above bars** = probability value (Student unpaired *t* test, 2-tailed). CHF = congestive heart failure; ESPVR = end-systolic pressure-volume relationship; other abbreviations as in Figure 2.

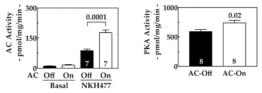

Figure 4. LV cAMP Production and PKA Activity

(Left) Increased AC_{VI} expression was associated with increased cAMP generation in response to NKH477 (10 μM) stimulation of LV samples. Basal cAMP generation was not changed by AC_{VI} expression. **(Right)** Increased cardiac AC_{VI} expression was associated with increased PKA activity in LV samples. **Numbers in bars** = animals studied; **bars** = mean values; **error bars** = 1 SE; **number above bars** = probability value (Student unpaired *t* test, 2-tailed). cAMP = cyclic adenosine monophosphate; PKA = protein kinase A; other abbreviations as in Figure 2.

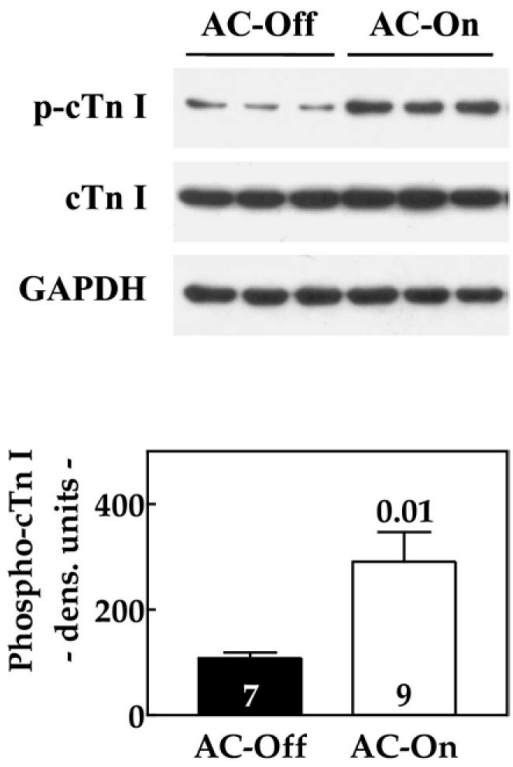

Figure 5. LV cTnI Phosphorylation

The Western blot shows a marked increase in cTnI phosphorylation in LV samples from mice 5 weeks after activation of AC_{VI} transgene expression. Summary of data from Western blotting is shown in the lower panel. **Numbers in bars** = animals studied; **bars** = mean values; **error bars** = 1 SE; **number above bars** = probability value (Student unpaired *t* test, 2-tailed). Abbreviations as in Figure 2.

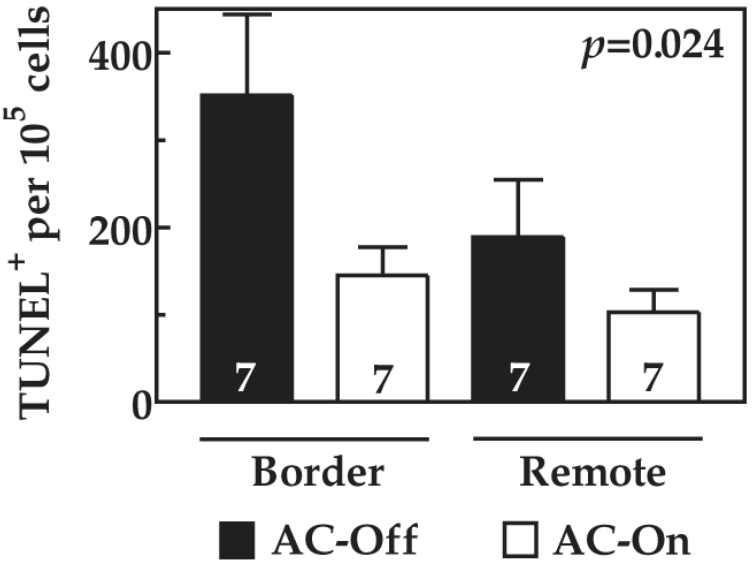

Figure 6. Apoptosis

Rates of LV apoptosis (TUNEL staining) were increased in both border and remote zones compared with the normal rate in murine LV of 50 TUNEL-positive nuclei per 100,000 cells (1). Activation of AC_{VI} expression reduced the rate of apoptosis by about 50% in both regions. **Numbers in bars** = animals studied; **bars** = mean values; **error bars** = 1 SE; p value = probability value for gene effect (2-way analysis of variance). TUNEL = terminal 2′-deoxyuridine 5′-triphosphate nick-end labeling; other abbreviations as in Figure 2.

J Am Coll Cardiol. Author manuscript; available in PMC 2011 May 16.

Table 1

Echocardiography: Effects of Increased AC_{VI} Expression

	AC-Off (n = 15)			AC-On (n = 19)			
	Before Rx	5 Weeks After Rx	Change	Before Rx	5 Weeks After Rx	Change	p Value
HR (beats/min)	394 ± 14	412 ± 15	18 ± 13	408 ± 11	430 ± 12	21 ± 17	0.88
EDD (mm)	5.5 ± 0.1	5.6 ± 0.1	0.1 ± 0.1	5.9 ± 0.2	5.9 ± 0.2	0.03 ± 0.1	0.70
ESD (mm)	4.8 ± 0.1	5.0 ± 0.2	0.2 ± 0.1	5.1 ± 0.2	5.0 ± 0.3	0.1 ± 0.1	0.17
PWd (mm)	0.66 ± 0.03	0.57 ± 0.02	−0.09 ± 0.03	0.64 ± 0.02	0.61 ± 0.02	−0.03 ± 0.02	0.14
IVSd (mm)	0.48 ± 0.02	0.47 ± 0.02	−0.01 ± 0.02	0.49 ± 0.03	0.48 ± 0.02	−0.02 ± 0.02	0.89
FAC (%)	13 ± 1	12 ± 1	−1 ± 1	13 ± 1	16 ± 1	3 ± 1	0.0001
LVEF (%)	24 ± 1	22 ± 1	−2 ± 1	24 ± 2	28 ± 2	5 ± 1	0.0001

Values represent mean ± SE. The p values are from the Student *t* test comparing changes in each parameter between groups (2-tailed, unpaired).

AC_{VI} = adenylyl cyclase VI; EDD = end-diastolic diameter; ESD = end-systolic diameter; FAC = fractional area change; HR = heart rate; IVSd = interventricular septal diastolic wall thickness; LVEF = left ventricular ejection fraction; PWd = posterior diastolic wall thickness; Rx = treatment.

Table 2

Basal Left Ventricular Function: Effects of Increased AC_{VI} Expression

	AC-Off (n = 9)	AC-On (n = 12)	p Value
HR (beats/min)	424 ± 34	452 ± 20	0.46
CO (ml/min)	4.0 ± 0.4	6.8 ± 1.0	0.029
ESPVR (mm Hg/μl)	2.4 ± 0.7*	10.1 ± 1.8*	0.014
LV +dP/dt (mm Hg/s)	5,178 ± 375	7,149 ± 551	0.013
LV −dP/dt (mm Hg/s)	−4,901 ± 459	−6,408 ± 505	0.046
EDP (mm Hg)	17 ± 2	12 ± 1	0.039
Tau (ms)	11 ± 1	7 ± 1	0.008

Values represent mean ± SE. p value is from Student *t* test comparing changes in each parameter between groups (2-tailed, unpaired).

* For ESPVR, AC-Off, n = 5; AC-On, n = 10.

CO = cardiac output; EDP = end-diastolic pressure; ESPVR = end-systolic pressure–volume relationship; HR = heart rate; LV = left ventricular.

Table 3

Echocardiography: Nontransgenic C57BL/6 Mice ± Doxycycline

	Doxycycline (n = 7)			No Doxycycline (n = 11)			
	5 Weeks After MI	10 Weeks After MI	Change	5 Weeks After MI	10 Weeks After MI	Change	p Value
HR (beats/min)	443 ± 20	459 ± 30	16 ± 31	464 ± 10	453 ± 24	−11 ± 18	0.43
EDD (mm)	4.9 ± 0.4	5.3 ± 0.5	0.5 ± 0.2	5.0 ± 0.3	5.3 ± 0.4	0.3 ± 0.2	0.46
ESD (mm)	4.3 ± 0.4	4.8 ± 0.6	0.6 ± 0.2	4.3 ± 0.4	4.7 ± 0.5	0.3 ± 0.2	0.52
PWd (mm)	0.6 ± 0.1	0.5 ± 0.1	−0.1 ± 0.1	0.6 ± 0.1	0.6 ± 0.05	−0.01 ± 0.04	0.81
IVSd (mm)	0.6 ± 0.1	0.6 ± 0.04	0.01 ± 0.04	0.6 ± 0.03	0.6 ± 0.02	−0.02 ± 0.03	0.61
FAC (%)	12 ± 2	11 ± 2	−0.6 ± 1.0	14 ± 2	14 ± 2	−0.1 ± 1.0	0.74
LVEF (%)	19 ± 4	20 ± 4	1 ± 2	22 ± 4	21 ± 4	−1.1 ± 1.5	0.32

Values represent mean ± SE. p value is from Student *t* test comparing changes in each parameter between groups (2-tailed, unpaired). Data from nontransgenic C57BL/6 mice (strain-matched). Experimental protocols were identical. Doxycycline was given continuously from 10 days before to 5 weeks after MI, or was not given (No Doxycycline group).

Abbreviations as in Table 1.

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