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PRECLINICAL RESEARCH

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

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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. (J Am Coll Cardiol 2008;51:1490–7) © 2008 by the American College of Cardiology Foundation

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 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

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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.

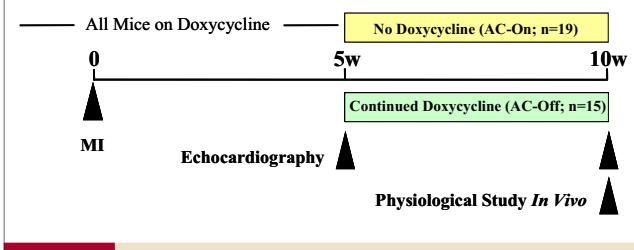

Figure 1 Experimental Design

See the text for a full description.
AC = adenylyl cyclase; MI = myocardial infarction.

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.

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

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 re-