Cardiac-Directed Adenylyl Cyclase Expression Improves Heart Function in Murine Cardiomyopathy

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Background—We tested the hypothesis that increased cardiac myocyte adenylyl cyclase (AC) content increases cardiac function and response to catecholamines in cardiomyopathy.

Methods and Results—Transgenic mice with cardiac-directed expression of AC type VI (ACVI) were crossbred with mice with cardiomyopathy induced by cardiac-directed Gq expression. Gq mice had dilated left ventricles, reduced heart function, decreased cardiac responsiveness to catecholamine stimulation, and impaired β-adrenergic receptor (βAR)–dependent and AC-dependent cAMP production. Gq/AC mice showed improved basal cardiac function in vivo (P=0.01) and ex vivo (P<0.0005). When stimulated through the βAR, cardiac responsiveness was increased (P=0.02), and cardiac myocytes showed increased cAMP production in response to isoproterenol (P=0.03) and forskolin (P<0.0001).

Conclusions—Increasing myocardial ACVI content in cardiomyopathy restores cAMP-generating capacity and improves cardiac function and responsiveness to βAR stimulation. (Circulation. 1999;99:3099-3102.)

Key Words: receptors, adrenergic, beta ■ gene therapy

A hallmark of dilated cardiomyopathy is decreased generation of cAMP by cardiac myocytes in response to β-adrenergic receptor (βAR) stimulation. However, treatments for clinical heart failure that increase myocardial cAMP content with pharmacological agents that stimulate the βAR or decrease the breakdown of cAMP generally have failed, perhaps because of deleterious consequences of unrelenting stimulation of the βAR. Indeed, overexpression of cardiac βARs in transgenic mice caused increased basal heart rate, function, and cAMP generation,1 and mice overexpressing cardiac Gq developed cardiomyopathy due to sustained βAR stimulation.2 Cardiac-directed overexpression of βARs failed to improve heart function and increased mortality in murine dilated cardiomyopathy.3

We recently showed that cardiac myocytes with increased expression of adenylyl cyclase (AC) produce more cAMP when stimulated through the βAR or AC.4 Cardiac-directed expression of AC type VI (ACVI) results in a phenotypically normal heart with normal basal function and cAMP levels but supranormal responses to catecholamine stimulation.5 Thus, receptor/G-protein overexpression and standard isotropic therapy yield continuous βAR activation and detrimental consequences, whereas overexpression of cardiac ACVI alters transmembrane signaling only when receptors are activated.

This could provide increased cAMP generation in heart failure in a manner that circumvents the deleterious consequences of sustained activation.

Cardiac-directed expression of Gq results in reduced left ventricular (LV) function, decreased cardiac responsiveness to catecholamines, and impaired βAR-dependent and AC-dependent cAMP production.6 The exact mechanism for dilation is unknown, but Gq is coupled to endothelin, angiotensin II, and α-adrenergic receptors, pathways that influence cardiac myocyte growth and remodeling. This model provides an opportunity to test the hypothesis that cardiac-directed AC expression can increase cAMP generation and restore heart function and response to catecholamines in dilated cardiomyopathy.

Methods

Animals

Animal use followed institutional guidelines. Generation of mice with cardiac-directed expression of murine ACVI was described recently,3 as was cardiac-directed Gq-induced cardiomyopathy.6 Gq-40 (FVB/N) mice, which show increased Gq protein expression and impaired systolic function compared with Gq-25 mice,6 were crossbred with ACVI (CB6) mice; transgene-negative siblings served as controls. Mice were studied at 15±4 weeks (range, 10 to 20 weeks). Transmural LV samples were fixed with formalin,
sectioned, and stained with hematoxylin and eosin and with Masson’s trichrome.

**Documentation of Transgene Expression**

Gene presence and expression was documented with polymerase chain reaction (not shown) and immunoblotting of cardiac homogenates with antibodies recognizing ACVI and Gq (Santa Cruz Biosciences) (Figure 1).4,5

**Echocardiography**

Animals were anesthetized with intraperitoneal injection of ketamine (50 mg/g) and thiobutabarbital (50 to 100 mg/g) and studied as previously described.5 Additional images were obtained after intraperitoneal injection of dobutamine (4 mg/g).

**Ex Vivo Heart Function**

Cardiac function in response to adrenergic stimulation was assessed in isolated perfused hearts (paced at 400 bpm, end-diastolic pressure 10 mm Hg) with an intraventricular balloon catheter to measure isovolumic LV pressure (previously described6). Dobutamine (0.001 to 100 μmol/L) was delivered in bolus doses at 5-minute intervals as LV pressure was recorded.

**Isolation of Cardiac Myocytes and cAMP Generation**

Ventricular myocytes were isolated.5 Equal numbers of viable cardiac myocytes were incubated (10 minutes, 25°C) in fresh DMEM containing no addition (basal), 10 μmol/L isoproterenol, or 10 μmol/L forskolin. Intracellular cAMP levels were determined by radioimmunoassay (Amersham Life Science).

**Myocardial βAR Number, G Proteins, G-Protein–Coupled Receptor Kinase Content, and Atrial Natriuretic Factor**

βAR density was estimated in radioligand binding experiments with [125I]-iodocyanopindolol (60 pmol/L). Polyclonal antibodies recognizing Gsa, Gia2, and G-protein–coupled receptor kinase 2 (GRK2) were used in immunoblots conducted on cardiac homogenates.4,5 Atrial natriuretic factor mRNA was evaluated as previously reported.6

**Statistics**

Data are reported as mean ± SEM. Group comparisons were made by ANOVA with Bonferroni correction. The primary intergroup comparison (Gq versus Gq/AC) was made with the Student t test (2-tailed).

**Results**

**Transgenic Mice**

We obtained substantial cardiac-directed expression of ACVI and Gq in the Gq/AC group and increased expression of Gq in the Gq line (Figure 1). The Table shows group characteristics. Litter sizes were normal, and mortality was invariant among the groups. LV histology showed no abnormalities.

**Echocardiography**

Basal and dobutamine-stimulated fractional shortening were reduced in the Gq mice. Concurrent expression of AC (Gq/AC) increased basal (P=0.01) and dobutamine-

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**Phenotypic Features**

<table>
<thead>
<tr>
<th>Feature</th>
<th>n</th>
<th>Control</th>
<th>Gq</th>
<th>Gq/AC</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td>EDD, mm</td>
<td>10</td>
<td>2.70±0.12</td>
<td>3.63±0.13†</td>
<td>3.53±0.14*</td>
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<tr>
<td>HR, bpm</td>
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<td>579±35</td>
<td>332±12‡</td>
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<tr>
<td>Dry HW, mg</td>
<td>11–16</td>
<td>24.5±1.7</td>
<td>26.6±1.2</td>
<td>25.1±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>(n=11)</td>
<td></td>
<td>(n=16)</td>
<td>(n=12)</td>
<td></td>
<td></td>
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<tr>
<td>Dry HW/BW, mg/g</td>
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<td>0.91±0.03</td>
<td>0.86±0.04‡</td>
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<tr>
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<td>(n=16)</td>
<td>(n=12)</td>
<td></td>
<td></td>
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<tr>
<td>βAR, cpm/μg</td>
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<td>23±4</td>
<td>27±4</td>
<td>26±1</td>
<td>NS</td>
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<tr>
<td>ANF, du</td>
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<td>10.0±2.1</td>
<td>3.6±0.5†</td>
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<tr>
<td>Gq, du</td>
<td>5</td>
<td>12.5±0.5</td>
<td>13.0±1.5</td>
<td>13.0±1.3</td>
<td>NS</td>
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<tr>
<td>Gq/AC, du</td>
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<td>GRK2, du</td>
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<td>11.8±0.7</td>
<td>12.2±0.8</td>
<td>12.6±1.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

EDD indicates end-diastolic dimension; HR, heart rate; HW, heart weight; BW, body weight; ANF, atrial natriuretic factor; du, arbitrary densitometry units; and n, number of animals per group.

*P<0.01, †P<0.001 (vs control), ‡P=0.01 (vs Gq). Values are mean±1 SEM.
AC production in response to isoproterenol (P<0.0001) and dobutamine-stimulated (P<0.01) fractional shortening (FS) differed between groups (ANOVA, P<0.0001 and P<0.01, respectively) and was reduced in Gq mice (G). Concurrent expression of AC (GA) increased basal (P<0.01) and dobutamine-stimulated (P<0.02) fractional shortening toward normal. Five animals per group. C indicates control. In all graphs, bars represent mean values and error bars denote 1 SEM. b, Ex vivo cardiac function. Peak positive and peak negative LV pressure development (LV dp/dt) in response to injections of dobutamine were measured in isolated perfused hearts from AC and Gq/AC animals. Concurrent expression of AC increased peak positive (P<0.0005) and peak negative LV dp/dt (P<0.04; data not shown), indicating increased LV contractility. Probability values are from 2-way ANOVA. Closed circles denote mean values from 5 Gq animals, open circles from 5 Gq/AC animals. c, Cardiac myocyte cAMP production: βAR stimulation. cAMP production by cardiac myocytes stimulated by 10 μmol/L isoproterenol differed between groups (P<0.002, ANOVA). Cardiac myocyte cAMP production was reduced in the Gq mice; concurrent expression of AC (Gq/AC) increased cAMP production (P<0.003). Five animals per group. d, Cardiac myocyte cAMP production: AC stimulation. Cardiac myocytes were stimulated by 10 μmol/L forskolin, and cAMP was measured. cAMP production differed between groups (P<0.001, ANOVA). Cardiac myocyte cAMP production was reduced in the Gq mice; concurrent expression of AC (Gq/AC) increased cAMP production (P<0.0001). Five animals per group.

Ex Vivo Heart Function
Concurrent expression of AC increased peak positive (P<0.0005; Figure 2b) and peak negative LV dp/dt (P<0.04), indicating increased rates of LV contractility and relaxation compared with Gq mice.

Transmembrane βAR Signaling
Gq mice showed reduced cardiac myocyte cAMP production, and concurrent AC expression (Gq/AC) increased cAMP production in response to isoproterenol (P<0.03) and forskolin (P<0.0001) (Figure 2c and 2d). Radioligand binding assays and immunoblotting indicated that βAR density and the contents of G proteins and GRK2 were unchanged (Table).

Discussion
We asked whether increased AC expression could favorably affect heart function in cardiomyopathy. Our data indicate that AC expression restores cAMP-generating capacity, improves basal heart function, and increases the heart’s response to βAR stimulation. These favorable effects did not decay over a broad age range (10 to 20 weeks), and hearts of AC/Gq mice showed no histological abnormalities. We have previously shown the safety and persistent favorable effects of life-long cardiac-directed AC expression.5 The mechanism for impaired βAR responsiveness in Gq cardiomyopathy is unknown but is associated with impaired cAMP production in cardiac membranes and a dilated poorly functioning heart, features that establish this as an apt model of clinical dilated cardiomyopathy, in which similar findings are present.

Increasing cardiac βAR expression and inhibition of GRK function have been examined as therapeutic interventions for heart failure.1 However, overexpression of the βAR worsened outcomes when concurrently expressed in murine cardiomyopathy and inhibition of GRK function completely prevented the development of cardiomyopathy.3 The persistence of chamber enlargement in the Gq/AC line, despite marked improvement in cardiac function and cAMP generation, is consonant with a treated condition. Had ACVI reversed this defect, one could infer that ACVI had simply prevented the heart failure phenotype from ever developing. Our data indicate the underlying cardiomyopathy is present but that the function of this diseased heart is substantially improved.

Are these findings relevant to the treatment of clinical dilated cardiomyopathy? The Gq cardiomyopathy model does not exhibit myocardial βAR downregulation, as seen in clinical heart failure. However, like failed human hearts, this model shows chamber enlargement, impaired systolic function, and diminished responsiveness to βAR stimulation in vivo, as well as decreased production of cAMP with βAR stimulation.

There are varying reports regarding whether forskolin-stimulated cAMP production is reduced in failing human myocardium,2,8 but a consistent finding is reduced βAR-stimulated cAMP generation,7 a finding that is also present in Gq cardiomyopathy.6 Overexpression of AC increases βAR-stimulated cAMP production even when βAR number and coupling and endogenous AC function and amount are normal.4,5 These data indicate that AC sets a limit on transmembrane βAR signaling in the heart and that increasing AC content is likely to increase transmembrane signaling independently of the endogenous amounts of βAR and AC.

Overexpression of the βAR, Gsa, or the use of inotropic drugs9 provides perpetual adrenergic activation with dire consequences. In contrast, AC overexpression provides increased recruitable adrenergic responsiveness without sustained adrenergic activation. This provides a rational potential therapeutic option for clinical dilated cardiomyopathy. In conclusion, increased cardiac AC content improves heart function and responsiveness to βAR stimulation in the setting of cardiomyopathy. This is associated with a restored ability of cardiac myocytes to generate cAMP in response to adrenergic stimulation.

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References
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