

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

David M. Roth, MD, PhD; Mei Hua Gao, PhD; N. Chin Lai, PhD; Jeff Drumm, BS; Nancy Dalton, BA; Jin Yao Zhou, BS; Jian Zhu, BS; Daniel Entrikin, BS; H. Kirk Hammond, MD

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 (AC_{VI}) were crossbred with mice with cardiomyopathy induced by cardiac-directed G_q expression. G_q mice had dilated left ventricles, reduced heart function, decreased cardiac responsiveness to catecholamine stimulation, and impaired β -adrenergic receptor (β AR)–dependent and AC-dependent cAMP production. G_q/AC mice showed improved basal cardiac function in vivo ($P=0.01$) and ex vivo ($P<0.0005$). When stimulated through the β AR, 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 AC_{VI} content in cardiomyopathy restores cAMP-generating capacity and improves cardiac function and responsiveness to β AR 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 (β AR) stimulation. However, treatments for clinical heart failure that increase myocardial cAMP content with pharmacological agents that stimulate the β AR or decrease the breakdown of cAMP generally have failed, perhaps because of deleterious consequences of unremitting stimulation of the β AR. Indeed, overexpression of cardiac β ARs in transgenic mice caused increased basal heart rate, function, and cAMP generation,¹ and mice overexpressing cardiac G_{sa} developed cardiomyopathy due to sustained β AR stimulation.² Cardiac-directed overexpression of β ARs 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 β AR or AC.⁴ Cardiac-directed expression of AC type VI (AC_{VI}) 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 inotropic therapy yield continuous β AR activation and detrimental consequences, whereas overexpression of cardiac AC_{VI} 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 G_q results in reduced left ventricular (LV) function, decreased cardiac responsiveness to catecholamines, and impaired β AR-dependent and AC-dependent cAMP production.⁶ The exact mechanism for dilation is unknown, but G_q is coupled to endothelin, angiotensin II, and α_1 -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 AC_{VI} was described recently,⁵ as was cardiac-directed G_q-induced cardiomyopathy.⁶ G_q-40 (FVB/N) mice, which show increased G_q protein expression and impaired systolic function compared with G_q-25 mice,⁶ were crossbred with AC_{VI} (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,

Received March 12, 1999; revision received April 12, 1999; accepted April 19, 1999.

From the Department of Medicine, VAMC-San Diego and University of California, San Diego (N.D., J.Z., H.K.H., D.E.); Department of Anesthesiology, VAMC-San Diego and University of California, San Diego (D.M.R.); and Collateral Therapeutics, Incorporated, San Diego, Calif (M.H.G., N.C.L., J.D., J.Y.Z., H.K.H.).

Drs Roth, Gao, and Lai contributed equally to this work.

Correspondence to H. Kirk Hammond, MD (111-A), VAMC-San Diego, 3350 La Jolla Village Dr, San Diego, CA 92161. E-mail khammond@ucsd.edu

Collateral Therapeutics is developing the use of an adenovirus-expressing AC_{VI} as a possible therapeutic agent for treating heart failure. Dr Hammond has a proprietary interest in Collateral Therapeutics.

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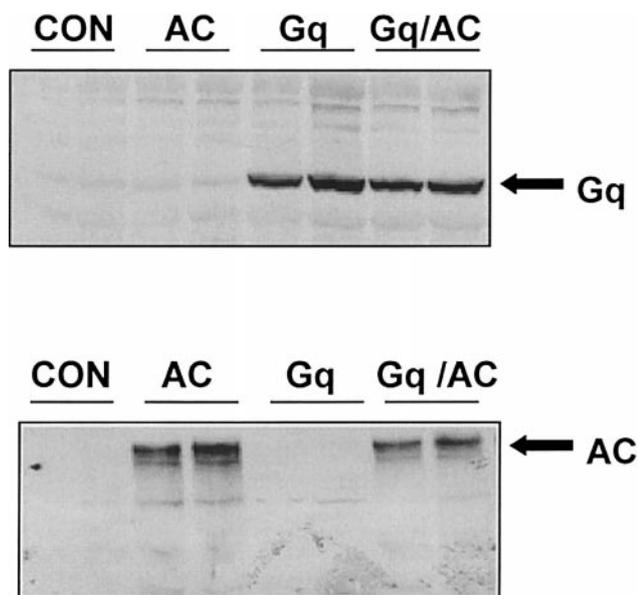


Figure 1. Western blot analysis. Top, With a polyclonal anti- G_q antibody, G_q protein was readily detectable in cardiac membrane homogenates obtained from G_q and G_q/AC transgenic mice but was barely detectable in hearts from transgene negative animals (CON) or AC transgenic mice. 100 μ g of protein per lane. Bottom, AC_{VI} protein was undetectable in cardiac homogenates from transgene negative mice (CON) and G_q -expressing mice but was abundant in hearts from AC transgenes and G_q/AC transgenes. 100 μ g of protein per lane.

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 AC_{VI} and G_q (Santa Cruz Biosciences) (Figure 1).^{4,5}

Echocardiography

Animals were anesthetized with intraperitoneal injection of ketamine (50 μ g/g) and thiobutabarbital (50 to 100 μ g/g) and studied as previously described.⁵ Additional images were obtained after intraperitoneal injection of dobutamine (4 μ g/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 μ mol/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 μ mol/L isoproterenol, or 10 μ mol/L forskolin. Intracellular cAMP levels were determined by radioimmunoassay (Amersham Life Science).

Myocardial β AR Number, G Proteins, G-Protein-Coupled Receptor Kinase Content, and Atrial Natriuretic Factor

β AR density was estimated in radioligand binding experiments with [¹²⁵I]-iodocyanopindolol (60 pmol/L). Polyclonal antibodies recognizing G_{sa} , $G_{i\alpha 2}$, 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 \pm SEM. Group comparisons were made by ANOVA with Bonferroni correction. The primary intergroup comparison (G_q versus G_q/AC) was made with the Student *t* test (2-tailed).

Results

Transgenic Mice

We obtained substantial cardiac-directed expression of AC_{VI} and G_q in the G_q/AC group and increased expression of G_q in the G_q 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 G_q mice. Concurrent expression of AC (G_q/AC) increased basal ($P=0.01$) and dobutamine-

Phenotypic Features

	n	Control	G_q	G_q/AC	ANOVA
EDD, mm	10	2.70 \pm 0.12	3.63 \pm 0.13†	3.53 \pm 0.14*	0.0001
HR, bpm	10	579 \pm 35	332 \pm 12†	417 \pm 36*	0.0001
Dry HW, mg	11–16	24.5 \pm 1.7	26.6 \pm 1.2	25.1 \pm 1.3	NS
		(n=11)	(n=16)	(n=12)	
Dry HW/BW, mg/g	11–16	0.97 \pm 0.03	0.91 \pm 0.03	0.86 \pm 0.04	NS
		(n=11)	(n=16)	(n=12)	
β AR, cpm/ μ g	3	23 \pm 4	27 \pm 4	26 \pm 1	NS
ANF, du	4	4.7 \pm 0.6	10.0 \pm 2.1	3.6 \pm 0.5‡	0.01
G_i , du	5	12.5 \pm 0.5	13.0 \pm 1.5	13.0 \pm 1.3	NS
G_s , du	5	12.2 \pm 0.8	12.5 \pm 0.1	12.9 \pm 0.1	NS
GRK2, du	5	11.8 \pm 0.7	12.2 \pm 0.8	12.6 \pm 1.3	NS

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 G_q). Values are mean \pm 1 SEM.

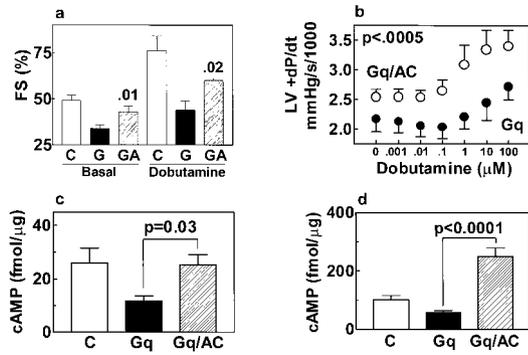


Figure 2. a, In vivo cardiac function. Basal ($P=0.0001$) and dobutamine-stimulated ($P=0.01$) fractional shortening (FS) differed between groups (ANOVA, $P=0.0001$ and $P=0.01$, respectively) and was reduced in G_q mice (G). Concurrent expression of AC (GA) increased basal ($P=0.01$) and dobutamine-stimulated ($P=0.02$) 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 G_q/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 G_q animals, open circles from 5 G_q/AC animals. c, Cardiac myocyte cAMP production: β AR stimulation. cAMP production by cardiac myocytes stimulated by 10 μ M isoproterenol differed between groups ($P=0.002$, ANOVA). Cardiac myocyte cAMP production was reduced in the G_q mice; concurrent expression of AC (G_q/AC) increased cAMP production ($P=0.03$). Five animals per group. d, Cardiac myocyte cAMP production: AC stimulation. Cardiac myocytes were stimulated by 10 μ M forskolin, and cAMP was measured. cAMP production differed between groups ($P=0.001$, ANOVA). Cardiac myocyte cAMP production was reduced in the G_q mice; concurrent expression of AC (G_q/AC) increased cAMP production ($P<0.0001$). Five animals per group.

stimulated ($P=0.02$) fractional shortening toward normal (Figure 2a). G_q mice had reduced heart rates, as previously reported,⁶ and concurrent AC expression (G_q/AC) increased heart rate toward normal (Table). End-diastolic diameter was increased by G_q expression and unaffected by concurrent AC expression (Table). Wall thickness was invariant between groups (not shown).

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 G_q mice.

Transmembrane β AR Signaling

G_q mice showed reduced cardiac myocyte cAMP production, and concurrent AC expression (G_q/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 β AR 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 β AR stimulation. These favorable effects did not decay over a broad age range (10 to 20 weeks), and hearts of AC/ G_q 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 β AR responsiveness in G_q 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 β AR expression and inhibition of GRK function have been examined as therapeutic interventions for heart failure.³ However, overexpression of the β AR 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 G_q/AC line, despite marked improvement in cardiac function and cAMP generation, is consonant with a treated condition. Had AC_{VI} reversed this defect, one could infer that AC_{VI} 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 G_q cardiomyopathy model does not exhibit myocardial β AR downregulation, as seen in clinical heart failure. However, like failed human hearts, this model shows chamber enlargement, impaired systolic function, and diminished responsiveness to β AR stimulation in vivo, as well as decreased production of cAMP with β AR stimulation.

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

Overexpression of the β AR, $G_s\alpha$, 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 β AR stimulation in the setting of cardiomyopathy. This is associated with a restored ability of cardiac myocytes to generate cAMP in response to adrenergic stimulation.

Acknowledgments

This research was supported by the Department of Veteran's Affairs (Dr Hammond), NIH 1P50 HL-53773-01 (Dr Hammond), NRSA HL-07444 (Dr Gao), FAERSCA (Dr Roth), and Collateral Therapeutics, Inc. We

thank Y.Y. Lai for technical support, Dr P. Haghghi for reviewing the histology, Drs Tamsin Kelly and Paul Insel for reviewing the manuscript, and Gerald Dorn for providing the transgenic G_q -40 mouse.

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Circulation. 1999;99:3099-3102

doi: 10.1161/01.CIR.99.24.3099

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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