Disruption of adenylyl cyclase type V does not rescue the phenotype of cardiac-specific overexpression of $G_{\alpha q}$ protein-induced cardiomyopathy

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1Division of Cardiovascular Medicine, University of California, Davis, 2Department of Veterans Affairs, Northern California Healthcare System, Mather, 3Department of Medicine, University of California, San Diego, and Veterans Affairs San Diego Healthcare System, San Diego, California; and 4Department of Neuroscience, Ewha Women’s University School of Medicine, Seoul, Korea

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Timofeyev V, Porter CA, Tuteja D, Qiu H, Li N, Tang T, Singapuri A, Han PL, Lopez JE, Hammond HK, Chiamvimonvat N. Disruption of adenylyl cyclase type V does not rescue the phenotype of cardiac-specific overexpression of $G_{\alpha q}$ protein-induced cardiomyopathy. Am J Physiol Heart Circ Physiol 299: H1459–H1467, 2010. First published August 13, 2010; doi:10.1152/ajpheart.01208.2009.—Adenylyl cyclase (AC) is the principal effector molecule in the β-adrenergic receptor pathway. $AC_{V}$ and $AC_{VI}$ are the two predominant isoforms in mammalian cardiac myocytes. The disparate roles among AC isoforms in cardiac hypertrophy and progression to heart failure have been under intense investigation. Specifically, the salutary effects resulting from the disruption of $AC_{V}$ have been established in multiple models of cardiomyopathy. It has been proposed that a continual activation of $AC_{V}$ through elevated levels of protein kinase C could play an integral role in mediating a hypertrophic response leading to progressive heart failure. Elevated protein kinase C is a common finding in heart failure and was demonstrated in murine cardiomyopathy from cardiac-specific overexpression of $G_{\alpha q}$ protein. Here we assessed whether the disruption of $AC_{V}$ expression can improve cardiac function, limit electrophysiological remodeling, or improve survival in the $G_{\alpha q}$ mouse model of heart failure. We directly tested the effects of gene-targeted disruption of $AC_{V}$ in transgenic mice with cardiac-specific overexpression of $G_{\alpha q}$ protein using multiple techniques to assess the survival, cardiac function, as well as structural and electrical remodeling. Surprisingly, in contrast to other models of cardiomyopathy, $AC_{V}$ disruption did not improve survival or cardiac function, limit cardiac chamber dilation, halt hypertrophy, or prevent electrical remodeling in $G_{\alpha q}$ transgenic mice. In conclusion, unlike other established models of cardiomyopathy, disrupting $AC_{V}$ expression in the $G_{\alpha q}$ mouse model is insufficient to overcome several parallel pathophysiological processes leading to progressive heart failure.

In a well-described transgenic mouse with cardiac-specific overexpression of the heterotrimeric G protein $\alpha_{q}$-subunit ($G_{\alpha q}$), an overexpression of $AC_{VI}$ attenuates hypertrophy, prevents heart failure, and reduces mortality (40), whereas an overexpression of $AC_{V}$ does not (45). Moreover, the disruption of $AC_{V}$ activity in murine models of age-related (48), isoproterenol-induced (29, 48), or pressure-overload cardiomyopathy (30, 31, 48) has been associated with salubrious effects to the heart. Indeed, the proposed development of novel pharmaceuticals specifically inhibiting $AC_{V}$ as a potential therapy for chronic heart failure underscores the relevance of this isoform (14).

Heart failure is one of the leading causes of morbidity and mortality. Once cardiac failure develops, the condition is generally irreversible and is associated with a high mortality rate. Therefore, the molecular mechanisms that initiate or precipitate the transition to heart failure have undergone intense investigation, and there is evidence to suggest that an elevation in protein kinase C (PKC) activity (15, 17, 36, 42) may play an important role in the transition to heart failure. Specifically, increased PKC activity has been reported to be involved in the pathogenesis of cardiac hypertrophy and progression to heart failure (6, 35).

Motivated by recent reports suggesting $AC_{V}$ inhibition as a possible therapeutic target in the treatment of heart failure, we directly tested whether the deletion of $AC_{V}$ would have beneficial effects in $G_{\alpha q}$-associated cardiomyopathy using comprehensive in vivo and in vitro studies. Transgenic mice with cardiac-specific overexpression of $G_{\alpha q}$ provide a clinically relevant model of heart failure with chamber dilation, decreased cardiac contractility, electrical remodeling, depressed β-AR function, and a high mortality rate. Moreover, $G_{\alpha q}$ transgenic mice exhibit an elevation of PKC level, approximately threefold compared with transgene-negative siblings (8). Furthermore, PKC is directly activated along the $G_{\alpha q}$ signaling pathway. Hence, we reasoned that transgenic mice with a cardiac-specific overexpression of $G_{\alpha q}$ would provide an ideal means to specifically test the roles of $AC_{V}$ in the progression toward cardiac failure.

THE DISPARATE ROLES among adenylyl cyclase (AC) isoforms in cardiac hypertrophy and progression to heart failure have been in focus for more than two decades. AC is the principal effector molecule along the β-adrenergic receptor (β-AR) pathway, and six ($AC_{II}, AC_{III}, AC_{V}, AC_{VI}, AC_{VII}$, and $AC_{VIII}$) of nine known isoforms are endogenous to the mammalian heart (33). Among these six isoforms, $AC_{V}$ and $AC_{VI}$ are the two predominant proteins in cardiac myocytes. A number of shared characteristics exist between $AC_{V}$ and $AC_{VI}$. They exhibit 65% amino acid homology (32) and are the only two AC isoforms that are inhibited by physiological Ca$^{2+}$ concentrations (4, 16). Similar to other isoforms, $AC_{V}$ and $AC_{VI}$ are sensitive to negative feedback by protein kinase A (PKA) (2, 20). $AC_{V}$ and $AC_{VI}$ (as well as $AC_{II}$) have been found localized primarily in caveolin-rich domains in cardiac fibroblasts (33). Despite similarities, the evidence indicates distinct physiological roles for $AC_{V}$ and $AC_{VI}$, as demonstrated in null-mutant mouse models of $AC_{V}$ vs. $AC_{VI}$ (25, 43, 44).

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MATERIALS AND METHODS

All animal care and procedures were approved by the University of California, Davis Institutional Animal Care and Use Committee. Animal use was in accordance with National Institutes of Health and institutional guidelines.

Generation of ACV gene-targeted mice. ACV knockout mice (ACV<sup>−/−</sup>) were generated as previously described (25, 44). The knockout mice were backcrossed with C57Bl/6J mice for greater than 10 generations. Two PCRs with primers specific for the mutated and wild-type alleles were used for genotype analysis. A previous study documented the absence of ACV mRNA expression in ACV<sup>−/−</sup> mouse hearts, which was associated with a significant decrease in cAMP production in cAMP production in left ventricular (LV) homogenates after isoproterenol stimulation (44).

Transgenic mice. ACV<sup>+/−</sup> mice (C57Bl/6J) were crossbred with mice with cardiac-directed expression of G<sub>q</sub> protein (G<sub>q</sub>-40 mice; FVB/N, provided by G. W. Dorn II, University of Cincinnati) (5, 47). Of note, the G<sub>q</sub> transgenic mice were backcrossed onto C57Bl/6J mice for greater than 10 generations before they were used for the crossbreeding to allow for the direct comparison of these lines without the confounding effects from differences in the background. Four lines emanating from this cross were studied: G<sub>q</sub>/ACV<sup>−/−</sup> (double positive), G<sub>q</sub> alone, ACV<sup>−/−</sup> alone, and control (double negative). Transgene incorporation into mouse DNA was confirmed using the PCR of tail tissue.

Analysis of cardiac function by echocardiography. M-mode measurements were used to assess systolic function as previously described (26). Measurements represent the average of twelve selected cardiac cycles from at least two separate scans performed in a random-blind manner with papillary muscles used as a point of reference to establish the consistency in level of the scan. End diastole was defined as the maximal LV diastolic dimension, and end systole was defined as the peak of posterior wall motion. Fractional shortening (FS), a measurement of systolic function, was calculated from LV diastolic dimension as follows: FS = [(EDD − ESD)/EDD] × 100%, where EDD and ESD are LV end-diastolic and end-systolic dimensions, respectively.

Western blot analysis. Immunoblots were performed as previously described (49). Anti-β-myosin heavy chain antibody (NOQ7.5.4D, Sigma) was used as an internal loading control.

Electrocardiograph recordings. ECG recordings were obtained using Bioamplifier (BMA 831, CWE, Ardmore, PA). Mice were placed on a temperature-controlled warming blanket at 37°C. Four consecutive 2-min time frames of ECG data were obtained from each mouse. Signals were low-pass filtered at 0.2 kHz and digitized using Digidata 1200 (Axon Instruments). A total of 100 beats were analyzed from each animal in a blinded manner.

Electrophysiology recordings from transgenic animals. Single ventricular myocytes were isolated from transgenic and double-negative (control) sibling mice from the same littermates at 8 to 9 mo of age. For action potential recordings, patch pipettes were backfilled with amphotericin (200 µg/ml). The pipette solution contained (in mM) 120 K<sup+</sup> glutamate, 25 KCl, 1 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, and 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (pH 7.4 with KOH).

For K<sup+</sup> current recording, the external solution contained 130 mM N-methylglucamine, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1 µM nimodipine, 10 mM glucose, and 10 mM HEPES (pH 7.4 with HCl).

The pipette solution contained (in mM) 140 KCl, 4 Mg-ATP, 1 MgCl<sub>2</sub>, 10 EGTA, and 10 HEPES (pH 7.4 with KOH).

Chemicals were purchased from Sigma Chemical (St. Louis, MO) unless stated otherwise. All experiments were performed using 3 M KCl agar bridges. Cell capacitance was calculated as the ratio of net charge (the integrated area under the current transient) to the magnitude of the pulse (20 mV). Currents were normalized to cell capacitance to obtain the current density. The series resistance was compensated electronically. In all experiments, a series resistance compensation of ≥90% was obtained. Currents were recorded using Axopatch 200B amplifier (Axon Instrument), filtered at 2 kHz using a four-pole Bessel filter, and digitized at a sampling frequency of 10 kHz. Data analysis was carried out using custom-written software and commercially available PC-based spreadsheet and graphics software (MicroCal Origin, version 7.0).

RNA isolation, reverse transcription, and quantitative PCR. Total RNA was isolated from LV free wall of mice with five different genotypes: wild-type, G<sub>q</sub> alone, G<sub>q</sub>/ACV<sup>−/−</sup>, ACV<sup>−/−</sup>, and cardiac-directed ACV<sub>1α</sub> overexpression (ACV<sub>1α</sub> transgenic), using TRIZol reagent (Invitrogen). Isolated RNA was subjected to DNase I (Invitrogen) treatment and subsequently to reverse transcription using Superscript III Reverse Transcriptase (Invitrogen), dNTPs, and oligo dT primers. Parallel reactions without the reverse transcription enzyme were also performed and used as controls to rule out the possibility of genomic DNA contamination.

Quantitative PCR (qPCR) was performed using Applied Biosystems Fast 7900HT real-time PCR system and RT<sup>2</sup> Fast SYBR Green/ROX qPCR master mix reagent (SA Biosciences). qPCR-specific primers were custom made from SA Biosciences corresponding to mouse accession numbers: NM_001012765 for ACV<sub>1α</sub>, NM_007405 for ACV<sub>1</sub>, and NM_008084 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The results were analyzed using Sequence Detection software (version 2.2.1). The quantity of ACV<sub>1α</sub> and ACV<sub>1</sub> transcripts were normalized to internal control GAPDH transcripts. qPCR was performed in triplicate to ensure quantitative accuracy.

Assessment of cAMP levels. cAMP levels in cardiac tissues from G<sub>q</sub> alone, G<sub>q</sub>/ACV<sup>−/−</sup>, ACV<sup>−/−</sup>, and control mice were measured using cAMP XF Assay Kit (Cell Signaling Technology, Danvers, MA) as per the manufacturer’s instructions. The hearts were rapidly excised and retrogradely perfused through the cannulation of aorta using phosphate-buffered saline to remove blood from the cardiac tissue. The tissue was then snap frozen using liquid nitrogen and stored at −80°C until use.

Survival. To compare the rates of survival between G<sub>q</sub> alone, G<sub>q</sub>/ACV<sup>−/−</sup> groups, a Kaplan-Meier cumulative curve was used. A log-rank test calculated the statistical significance of differences between the two groups. The longevity of ACV<sup>−/−</sup> and double-negative control mice exceeded the duration of the experiment and were excluded from survival analysis.

Statistical analysis. Data were presented as means ± SE where appropriate. An analysis of statistical significance was calculated using SigmaStat software. For multiple comparisons, one-way analysis of variance combined with Dunnett’s test was used. The null hypothesis was rejected when P < 0.05 (two tailed).

RESULTS

ACV<sub>1α</sub> disruption does not prevent cardiac hypertrophy or chamber dilation in G<sub>q</sub> transgenic mice. Figure 1A shows photomicrographs of examples of whole hearts from ACV<sub>1α</sub>, G<sub>q</sub>, G<sub>q</sub>/ACV<sup>−/−</sup>, and control mice at 6 mo of age. As expected, G<sub>q</sub> transgenic mice exhibit evidence of cardiomyopathy with chamber dilation. On the other hand, ACV<sub>1α</sub> animals show normal chamber size compared with control mice. More importantly, in contrast to other models of heart failure, the deletion of ACV<sub>1α</sub> does not rescue the cardiomyopathic phenotype in G<sub>q</sub> transgenic animals. Figure 1B shows a significant increase in cell capacitance of LV myocytes.
isolated from both G\textsubscript{eq} and G\textsubscript{eq}/AC\textsubscript{V}\textsuperscript{−/−} animals compared with AC\textsubscript{V}\textsuperscript{−/−} and control groups at 6 mo of age. Figure 1C displays summary data for the heart, liver, and lung weight, normalized to body weight, illustrating a significant increase in the heart weight-to-body weight ratio in both G\textsubscript{eq} and G\textsubscript{eq}/AC\textsubscript{V}\textsuperscript{−/−} animals compared with controls. Figure 1D illustrates the histologic sections with hematoxylin and eosin stain, comparing the four groups of animals. Both G\textsubscript{eq} and G\textsubscript{eq}/AC\textsubscript{V}\textsuperscript{−/−} hearts show dilatation of all four cardiac chambers.

Echocardiography was used to directly assess cardiac function in the four groups of animals. Figure 1E shows examples of M-mode echocardiogram displaying chamber dilatation and reduced FS in G\textsubscript{eq} and G\textsubscript{eq}/AC\textsubscript{V}\textsuperscript{−/−} transgenic mice. Summary data for the FS, ESD, and EDD are shown in Table 1. The
Disruption of ACV does not improve cardiac function in Gαq/ACV−/− transgenic mice.

To further confirm the lack of the beneficial effects of ACV deletion in Gαq mice, we tested for the induction of a fetal gene, β-MHC isofrom, a well-documented hypertrophic marker (3) using Western blot analysis. GAPDH was used as a loading control. As expected, β-MHC is expressed only in Gαq transgenic mice but not in control or ACV−/− animals. Consistent with the functional analysis, Gαq/ACV−/− mice show a persistent expression of the β-MHC protein (Fig. 1F).

ACV disruption does not prevent sinus bradycardia in Gαq transgenic mice. We have previously documented that cardiac-directed expression of Gαq protein resulted in sinus bradycardia (47). Gαq and Gαq/ACV−/− mice show sinus bradycardia with a significant prolongation of the R-R intervals (*P < 0.05 compared with control animals, Fig. 1H). In contrast, ACV−/− mice demonstrate an increase in basal heart rates compared with control animals (*P < 0.05, Fig. 1G). There was no difference between Gαq and Gαq/ACV−/− animals.

Assessment of ACV and ACVβ expression level in ACV and Gαq/ACV−/− mice. The absence of the beneficial effects from the deletion of ACV in Gαq-mediated cardiomyopathy compared with other previous published data in murine models of age-related (48), isoproterenol-induced (29, 48), or pressure-overload cardiomyopathy (30, 31, 48) has prompted us to examine the expression level of ACV in Gαq and Gαq/ACV−/− mice (Fig. 2A). Moreover, to further ensure that ACV knockout animals are specific for ACV isofrom, we directly assessed the transcript level of ACVβ1 in ACV−/− and Gαq/ACV−/− mice (Fig. 2B). Previously described cardiac-directed overexpression of ACVβ1 transgenic animals was used as a control for the assessment of the ACVβ1 transcript. Figure 2A demonstrates that the ACV transcript is completely absent in ACV−/− and Gαq/ACV−/− animals as expected. Moreover, ACV and ACVβ1 transcript levels are only slightly

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Values are means ± SE; n, number of mice. ACV, adenylyl cyclase type V; ESD, left ventricular end-systolic dimension; EDD, left ventricular end-diastolic dimension; FS, fractional shortening, calculated from left ventricle dimensions as follows: FS = [(EDD − ESD)/EDD] × 100%. *P < 0.05 compared with control.
DISRUPTION OF ADENYLYL CYCLASE TYPE V IN CARDIOMYOPATHY

Elevated (~1.5-fold) in G_{aq} transgenic animals compared with controls (Fig. 2, A and B). The ACV_{I} transcript level is also slightly elevated (~1.8-fold) in ACV~^{−/−}~ animals, supporting the previous finding that the ACV knockout mouse is specific for the ACV isoform. The increase in the ACV_{I} transcript level in ACV~^{−/−}~ animals may be secondary to compensatory responses in the knockout animals. Finally, as expected, the ACV_{I} transcript level is significantly elevated (>110-fold) in ACV_{I} transgenic animals compared with controls.

Assessment of cAMP level in ACV and G_{aq}/ACV~^{−/−}~ mice. We next examined the cAMP level in the cardiac tissues from four groups of animals (Fig. 2C). cAMP levels in G_{aq} or ACV~^{−/−}~ animals show a trend toward a decrease compared with control, but the differences are not statistically significant. On the other hand, G_{aq}/ACV~^{−/−}~ exhibits a significant decrease in cAMP level compared with control. The data support the notion that the disruption of ACV in G_{aq} transgenic animals results in a decrease in cAMP level without rescuing the cardiomyopathic phenotypes.

ACV disruption does not prevent electrical remodeling in G_{aq} transgenic mice. Previous studies have documented that G_{aq}-induced cardiomyopathy is associated with electrical remodeling with a prolongation of cardiac action potential (47). Here we tested whether the deletion of ACV may prevent electrical remodeling in G_{aq} transgenic animals. Figure 3, A–D, shows recordings of action potentials from cardiomyocytes isolated from LV free wall in each of the four groups. Action potentials at 50 and 90% of repolarization (APD_{50} and APD_{90}, respectively) recorded from both the G_{aq} and G_{aq}/ACV~^{−/−}~ groups demonstrate significant prolongation compared with those of control animals. However, there was no significant difference between the G_{aq} and G_{aq}/ACV~^{−/−}~ groups.

We have previously shown that cardiac action potential prolongation in G_{aq} transgenic animals results, at least in part, from a decrease in the outward K~^{+}~ current and the inward rectifier K~^{+}~ current (I_{K1}) and that the salubrious effects of ACV_{I} overexpression in G_{aq} proteins transgenic mice are associated with the upregulation of both the outward K~^{+}~ current and I_{K1} (47). Here we directly examined the effects of ACV disruption on the K~^{+}~ current in the G_{aq} cardiomyopathy model. Figure 4, A–D, demonstrates examples of outward K~^{+}~ current recorded from each of the four groups. The G_{aq} and G_{aq}/ACV~^{−/−}~ groups show a significant decrease in both the transient outward K~^{+}~ current (I_{to}) and the sustained K~^{+}~ current densities (Fig. 4, B and D). Summary data for I_{to} and the sustained K~^{+}~ current densities are presented in Fig. 4, C and F, respectively. There was no difference between the recorded densities of I_{to} and the sustained outward K~^{+}~ current from the G_{aq} and G_{aq}/ACV~^{−/−}~ groups. Similarly, I_{K1} within the G_{aq} and G_{aq}/ACV~^{−/−}~ groups were very similar and significantly smaller than the current densities in ACV~^{−/−}~ and control animals (Fig. 5).

ACV disruption did not improve survival in G_{aq} transgenic mice. Finally, Kaplan-Meier mortality analyses were performed comparing the G_{aq} and G_{aq}/ACV~^{−/−}~ animals (Fig. 6). There were no significant differences in the survival between the two groups of animals, consistent with our data showing a lack of improvement in cardiomyopathy and electrical remodeling in G_{aq} transgenic animals by the disruption of ACV (P = 0.349).

**DISCUSSION**

The present study directly tested the possible beneficial effects of gene-targeted disruption of ACV in transgenic mice with cardiac-specific overexpression of G_{aq} protein using multiple techniques to assess the survival, cardiac function, as well as structural and electrical remodeling. Our study provides new evidence that, in contrast to other models of cardiomyopathy, ACV disruption did not improve survival or cardiac function, limit cardiac chamber dilation, or prevent electrical remodeling in G_{aq} transgenic mice. Indeed, the findings are relevant and important in view of the proposed development of novel pharmaceuticals specifically inhibiting ACV as a potential therapy for chronic heart failure.

Fig. 3. ACV disruption does not prevent action potential (AP) prolongation in G_{aq} transgenic mice. A–D: examples of AP recordings from transgenic mice compared with control littermates. AP recordings from G_{aq} and G_{aq}/ACV~^{−/−}~ transgenic mice were significantly prolonged compared with control littermates. There were no statistical differences in the length of APs between G_{aq}/ACV~^{−/−}~ and G_{aq} transgenic mice. E: summary data showing AP duration at 50 and 90% repolarization (APD_{50} and APD_{90}, in ms). *P < 0.05 compared with control animals; n = 30 to 50 cells for each group.
AC expression and its implications in cardiomyopathy. The initially attractive idea of restoring cardiac contractility in heart failure by stimulating $\beta$-AR pathways has not been shown to reduce mortality. Indeed, the administration of dobutamine (23), a $\beta_1$-AR agonist, or milrinone (34), a phosphodiesterase inhibitor, in clinical trials of patients with severe heart failure resulted in increases in mortality. Similarly, cardiac-directed expression of $G_{q}$, $\beta_1$-AR, or $\beta_2$-AR proteins in murine models Fig. 4. ACV disruption does not prevent $K^+$ current downregulation in $G_{q}\alpha$ transgenic mice. $Ca^{2+}$-independent outward $K^+$ current density from 4 different groups of animals (A, B, D, and E). Examples of current traces elicited from a holding potential of $-80$ mV using test potentials in duration of 2.5 s from $-70$ to $+60$ mV along 10-mV increments. C: summary data for the density of the peak outward components ($^*P < 0.05$ comparing $G_{q}/ACV^{-/-}$ and control mice; and $^*P < 0.05$ comparing $G_{q}$ and control mice). F: summary data for the density of the sustained components (measured at the end of the pulse, $^*P < 0.05$ comparing $G_{q}$ and $G_{q}/ACV^{-/-}$ with control mice; $n = 12$–16 cells for each group). V, voltage.

Fig. 5. ACV disruption does not prevent the downregulation of the inward rectifier $K^+$ current ($I_{K1}$) in $G_{q}\alpha$ transgenic mice. A: examples of current traces recorded from a holding potential of $-80$ mV using test potentials in duration of 2.5 s from $-130$ to $+60$ mV in 10-mV increments in control. B: after applying BaCl$_2$. C: after applying the BaCl$_2$-sensitive current. D: summary data for the BaCl$_2$-sensitive current density ($I_{K1}$ density) in single free wall LV myocytes isolated from the 4 groups. Inset: outward $I_{K1}$ component from each group for further clarity ($^*P < 0.05$; $n = 8$–15 for each group).
all demonstrate sustained increases in heart rate, decreased contractility, cardiomyopathy, and increased mortality (7, 9, 11, 21, 27, 39).

Remarkably, unlike the overexpression or stimulation of β-ARs, the overexpression of AC does not progress toward cardiac hypertrophy. The overexpression of ACV prevents heart failure in the Gαq transgenic model (40) and, additionally, does not affect the basal heart rate or heart function compared with control animals (47). This rescue is associated with a restoration of heart function, the prevention of electrical remodeling, and a marked reduction in the mortality. The mechanisms underlying the observed beneficial effects remain unclear, but several proposals exist (37).

The increased expression of ACV, in contrast to ACVI, does not prevent cardiac hypertrophy in the Gαq model of cardiomyopathy (45). ACV overexpression either slightly increases heart rate (46) in basal conditions or has no effects (10). On the other hand, the disruption of ACV has demonstrated benefits in several animals of cardiomyopathy. In the age-related model, older control mice have a greater disposition toward cardiomyocyte apoptosis, fibrosis, and decreased longevity compared with ACV knockout mice (48). Data taken from the pressure-overload model show that the LV ejection fraction (29, 30) improves in ACV knockout compared with control mice. Furthermore, this improvement is associated with reduced cardiac apoptosis in the ACV knockout compared with control mice (48). An ablation of ACV with a subsequent isoproterenol infusion results in an increased expression of Bcl-2, a suppressor of apoptosis in the Akt signaling pathway (31). Indeed, ACV has been viewed as a potential therapeutic target for chronic heart failure (14).

Our data indicate that the role of the ACV isoform may be diminished in Gαq-mediated cardiomyopathy compared with other animal models of heart failure. The beneficial effects associated with ACVΔI overexpression in the Gαq model cannot be explained by suppression of ACV because an absence of ACV did not rescue the phenotype. The reported beneficial effects from disrupting ACV expression were not present in our experiments, and this absence distinguishes Gαq overexpression from a set of cardiomyopathy models.

The absence of benefits from the deletion of ACV in Gαq-mediated cardiomyopathy prompted a closer examination of cardiomyopathy models. The models of cardiomyopathy can be arranged from pronounced to subtle salutary effects from ACV ablation as follows: isoproterenol induced, age related, pressure overload, and Gαq mediated. The disruption of ACV in isoproterenol-induced cardiomyopathy results in improved FS, decreased cardiac mass, and reduced cardiomyocyte apoptosis compared with those in control mice (44). In age-related decline in LV diastolic function, disrupting ACV decreased cardiomyocyte apoptosis and transgenic mice lived 30% longer than sibling controls (50). Pressure-overloaded ACVΔI′ mice show less cardiomyocyte apoptosis than control animals (30), though the disruption of ACV in aortic banding does not prevent the progression to hypertrophy (48).

Administering isoproterenol to control mice produced a slight elevation of PKC expression; however, this effect was diminutive compared with PKA activation (36). Disrupting the PKA regulatory subunit-RIIβ in mice increases longevity and reduces body size, mirroring the beneficial effects of disrupting ACV in age-related cardiomyopathy (38). PKA attenuation has been suggested as part of a mechanism for the observed benefits of ACV disruption in age-related cardiomyopathy (50). Constitutively active PKCε parallels the progression to cardiomyopathy with age (13), but the intrinsic elevation of PKC has only been hypothesized in age-related cardiomyopathy. Elevated PKC activity in pressure overload has been reported (15, 17, 42), in particular, PKCα and PKCβ but not PKCε (1). Although the rising levels of catecholamine in the blood plasma (12, 41) are suggestive of downstream AC activation, PKA activation precedes hypertrophy in aortic banding and is not clearly linked with the development of hypertrophy (24). Overexpressing Gαq leads to a prominent elevation in PKC levels (particularly PKCα) (7). PKCα can stimulate ACV [isoforms ζ (22), α (18), and γ (18) interact with ACV], but our results show that cardiac hypertrophy manifests despite any possible interaction between PKC and ACV.

Outcomes of disrupting ACV in the Gαq transgenic model of cardiomyopathy. Electrical remodeling is well documented in several models of heart failure including human heart failure. The downregulation of K+ channel expression is common to cardiac hypertrophy and failure and is associated with a prolonged action potential. A prolonged action potential, in turn, may be involved in the pathogenesis of cardiac hypertrophy through increasing intracellular Ca2+ entering mostly through L-type Ca2+ channels during the plateau phase. Our previous data demonstrate that the beneficial effects of ACVΔI overexpression in the Gαq transgenic model are associated with the prevention of electrical remodeling of outward K+ current and IK1 (47). ACV deletion, however, does not reproduce these beneficial effects in the Gαq transgenic model; outward K+ current and IK1 remain unchanged between Gαq and GαqΔI′ ACVΔI′ mice. The two groups of animals, Gαq and GαqΔI′ ACVΔI′, displayed a similar prolongation of the action potentials and sinus bradycardia. The mechanisms of increase mortality in Gαq and GαqΔI′ ACVΔI′ mice may be related to the documented prolongation in APD and possibly brady- or tachyarrhythmias. However, other mechanisms may be involved including progressive heart failure.
Interestingly, the ACV−/− group shows a slight, but statistically significant, decrease in R-R interval compared with the control animals. A minor deviation in heart rates in ACV−/− mice compared with the control animals has previously been reported (28, 29, 43). A decreasing effect of acetylcholine receptors in ACV−/− mice compared with control mice was proposed to explain this deviation (28). The activation of the parasympathetic system as an explanation for heart rate deviations between ACV−/− and control mice appears reasonable since acetylcholine receptors have been found to be colocalized with AC in macromolecular complexes in cardiomyocytes (19).

**Conclusion.** Our results demonstrate that unlike in age-related, isoproterenol-induced, and pressure-overload models, a disruption of ACV in the Gαq transgenic model provides no benefits in preventing the development of cardiomyopathy. The disruption of the constant activation of ACV by PKC in Gαq transgenic mouse model may be insufficient to overcome several parallel pathophysiological processes leading to progressive heart failure.

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