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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The disparate roles among adenylyl cyclase (AC) isoforms in cardiac hypertrophy and progression to heart failure have been under intense investigation. Specifically, the salutary effects resulting from the disruption of ACV have been established in multiple models of cardiomyopathy. It has been proposed that a continual activation of ACV 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 ACV 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 ACV 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, ACV 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 ACV expression in the $G_{\alpha q}$ mouse model is insufficient to overcome several parallel pathophysiological processes leading to progressive heart failure.

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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. ACV and ACVI 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 ACV have been established in multiple models of cardiomyopathy. It has been proposed that a continual activation of ACV 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 ACV 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 ACV 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, ACV 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 ACV expression in the $G_{\alpha q}$ mouse model is insufficient to overcome several parallel pathophysiological processes leading to progressive heart failure.

Affiliation

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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 crossed with mice with cardiac-directed expression of G<sub>q</sub> protein (G<sub>q</sub>/40; mouse; 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> 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 dimensional changes 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 (AB1005-5.4D, Sigma) was used at 1:10,000 dilution. This antibody was found to be specific for β-myosin heavy chain (β-MHC) through a screening of different commercially available antibodies. Anti-GAPDH antibody (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 littersmates at 8 to 9 mo of age. All experiments were performed using the conventional whole cell patch-clamp technique at room temperature.

For action potential recordings, patch pipettes were backfilled with amphoterin (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).

The extracellular solution contained (in mM) 138 NaCl, 4 KCl, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 0.33 NaH<sub>2</sub>PO<sub>4</sub>, 10 glucose, and 10 HEPES (pH 7.4 with NaOH).

For K<sup>+</sup> current recording, the extracellular solution contained 130 mM N-methylglucamine, 5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1 μM nifedipine, 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>1</sub> overexpression (ACV<sub>1</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> 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, 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 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>/ACV<sup>−/−</sup>, ACV<sup>−/−</sup>, and control mice were measured using cAMP XP 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>/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 are 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 disruption does not prevent cardiac hypertrophy or chamber dilation in G<sub>q</sub>/ACV<sup>−/−</sup> transgenic mice. Figure 1A shows photomicrographs of examples of whole hearts from ACV<sup>−/−</sup>, G<sub>q</sub>/ACV<sup>−/−</sup>, and control mice at 6 mo of age. As expected, G<sub>q</sub>/ACV<sup>−/−</sup> transgenic mice exhibit evidence of cardiomyopathy with chamber dilation. On the other hand, ACV<sup>−/−</sup> animals show normal chamber size compared with control mice. More importantly, in contrast to other models of heart failure, the deletion of ACV does not rescue the cardiomyopathic phenotype in G<sub>q</sub>/ACV<sup>−/−</sup> transgenic animals. Figure 1B shows a significant increase in cell capacitance of LV myocytes.
isolated from both Gq and Gq/ACV-/- animals compared with ACV-/- 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 Gq and Gq/ACV-/- transgenic animals compared with controls. Figure 1D illustrates the histologic sections with hematoxylin and eosin stain, comparing the four groups of animals. Both Gq and Gq/ACV-/- 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 Gq and Gq/ACV-/- transgenic mice. Summary data for the FS, ESD, and EDD are shown in Table 1. The
disruption of AC\textsubscript{V} does not improve cardiac function in G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} transgenic mice.

To further confirm the lack of the beneficial effects of AC\textsubscript{V} deletion in G\textsubscript{aq} 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 G\textsubscript{aq} transgenic mice but not in control or AC\textsubscript{V}\textsuperscript{−/−} animals. Consistent with the functional analysis, G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} mice show a persistent expression of the β-MHC protein (Fig. 1F).

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

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

**Table 1. Summary of echocardiographic data in control, AC\textsubscript{V\textsubscript{I}}−/−, G\textsubscript{aq\textsubscript{I}} and G\textsubscript{aq}/AC\textsubscript{V\textsubscript{I}}−/− transgenic mice**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>G\textsubscript{aq\textsubscript{I}}</th>
<th>AC\textsubscript{V\textsubscript{I}}−/−</th>
<th>G\textsubscript{aq}/AC\textsubscript{V\textsubscript{I}}−/−</th>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Systolic posterior wall thickness, mm</td>
<td>1.5 ± 0.04</td>
<td>1.6 ± 0.09</td>
<td>1.5 ± 0.04</td>
<td>1.6 ± 0.08</td>
</tr>
<tr>
<td>End-diastolic posterior wall thickness, mm</td>
<td>1.1 ± 0.07</td>
<td>1.3 ± 0.08</td>
<td>1.1 ± 0.03</td>
<td>1.4 ± 0.01</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>1.3 ± 0.01</td>
<td>1.9 ± 0.12*</td>
<td>1.4 ± 0.07</td>
<td>1.8 ± 10.1*</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>2.6 ± 0.02</td>
<td>3.0 ± 0.11</td>
<td>2.8 ± 0.08</td>
<td>2.9 ± 0.12</td>
</tr>
<tr>
<td>FS, %</td>
<td>52.6</td>
<td>36.4*</td>
<td>53.6</td>
<td>37.2*</td>
</tr>
</tbody>
</table>

*Values are means ± SE; n, number of mice. AC\textsubscript{V\textsubscript{I}}, 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.

![Fig. 2. A and B: AC\textsubscript{V} and AC\textsubscript{V\textsubscript{I}} transcript levels normalized to GAPDH from control, G\textsubscript{aq\textsubscript{I}}, G\textsubscript{aq}/AC\textsubscript{V\textsubscript{I}}−/−, AC\textsubscript{V\textsubscript{I}}−/−, and cardiac-directed expression of AC\textsubscript{V\textsubscript{I}} transgenic animals. *P < 0.05 compared with control animals. C: cAMP concentrations (in nM) in cardiac tissues from G\textsubscript{aq\textsubscript{I}}, G\textsubscript{aq}/AC\textsubscript{V\textsubscript{I}}−/−, and AC\textsubscript{V\textsubscript{I}}−/− compared with control. *P ≤ 0.05 compared with control; n = 4 for each group.](http://ajpheart.physiology.org/DownloadedFrom)
elevated (~1.5-fold) in G\textsubscript{aq} transgenic animals compared with controls (Fig. 2, A and B). The AC\textsubscript{VI} transcript level is also slightly elevated (~1.8-fold) in AC\textsubscript{VI}\textsuperscript{−/−} animals, supporting the previous finding that the AC\textsubscript{V} knockout mouse is specific for the AC\textsubscript{V} isoform. The increase in the AC\textsubscript{VI} transcript level in AC\textsubscript{V}\textsuperscript{−/−} animals may be secondary to compensatory responses in the knockout animals. Finally, as expected, the AC\textsubscript{V}I transcript level is significantly elevated (>110-fold) in AC\textsubscript{V}I transgenic animals compared with controls.

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

 AC\textsubscript{V} disruption does not prevent electrical remodeling in G\textsubscript{aq} transgenic mice. Previous studies have documented that G\textsubscript{aq}-induced cardiomyopathy is associated with electrical remodeling with a prolongation of cardiac action potential (47). Here we tested whether the deletion of AC\textsubscript{V} may prevent electrical remodeling in G\textsubscript{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\textsubscript{50} and APD\textsubscript{90}, respectively) recorded from both the G\textsubscript{aq} and G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} groups demonstrate significant prolongation compared with those of control animals. However, there was no significant difference between the G\textsubscript{aq} and G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} groups.

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

 AC\textsubscript{V} disruption did not improve survival in G\textsubscript{aq} transgenic mice. Finally, Kaplan-Meier mortality analyses were performed comparing the G\textsubscript{aq} and G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} 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\textsubscript{aq} transgenic animals by the disruption of AC\textsubscript{V} (P = 0.349).

 DISCUSSION

 The present study directly tested the possible beneficial effects of gene-targeted disruption of AC\textsubscript{V} in transgenic mice with cardiac-specific overexpression of G\textsubscript{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, AC\textsubscript{V} disruption did not improve survival or cardiac function, limit cardiac chamber dilation, or prevent electrical remodeling in G\textsubscript{aq} transgenic mice. Indeed, the findings are relevant and important in view of the proposed development of novel pharmaceuticals specifically inhibiting AC\textsubscript{V} as a potential therapy for chronic heart failure.

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Fig. 3. AC\textsubscript{V} disruption does not prevent action potential (AP) prolongation in G\textsubscript{aq} transgenic mice. A–D: examples of AP recordings from transgenic mice compared with control littermates; AP recordings from G\textsubscript{aq} and G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} transgenic mice were significantly prolonged compared with control littermates. There were no statistical differences in the length of APs between G\textsubscript{aq}/AC\textsubscript{V}\textsuperscript{−/−} and G\textsubscript{aq} transgenic mice. E: summary data showing AP duration at 50 and 90% repolarization (AP\textsubscript{50} and AP\textsubscript{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 β-AR pathways has not been shown to reduce mortality. Indeed, the administration of dobutamine (23), a β1-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 Gαs, β1-AR, or β2-AR proteins in murine models...

ACV disruption does not prevent K⁺ current downregulation in Gαq transgenic mice. Ca²⁺-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 Gαq/ACV−/− and control mice; and *P < 0.05 comparing Gαq and control mice). F: summary data for the density of the sustained components (measured at the end of the pulse, *P < 0.05 comparing Gαq and Gαq/ACV−/− with control mice; n = 12–16 cells for each group). V, voltage.

ACV disruption does not prevent the downregulation of the inward rectifier K⁺ current (I_K1) in Gαq 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 BaCl₂. C: after applying the BaCl₂-sensitive current. D: summary data for the BaCl₂-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 ACV1 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 ACV1 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 isofrom may be diminished in Gαq-mediated cardiomyopathy compared with other animal models of heart failure. The beneficial effects associated with ACV1 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 ACV−/− 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-RIβ 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 PKCa 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 PKCδ 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 [isofoms 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 ACV1 overexpression in the Gαq transgenic model are associated with the prevention of electrical modeling of outward K+ current and I K1 (47). ACV deletion, however, does not reproduce these beneficial effects in the Gαq transgenic model; outward K+ current and I K1 remain unchanged between Gαq and Gαq/ACV−/− mice. The two groups of animals, Gαq and Gαq/ACV−/−, displayed a similar prolongation of the action potentials and sinus bradycardia. The mechanisms of increase mortality in Gαq and Gαq/ACV−/− 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\textsuperscript{−/−} 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\textsuperscript{−/−} mice compared with the control animals has previously been reported (28, 29, 43). A decreasing effect of acetylcholine receptors in ACV\textsuperscript{−/−} 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\textsuperscript{−/−} 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 G\textsubscript{aq} transgenic model provides no benefits in preventing the development of cardiomyopathy. The disruption of the constant activation of ACV by PKC in pressure-overloaded neonatal rat myocardium of an adaptive physiologic response. No conflicts of interest, financial or otherwise, are declared by the author(s).

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DISCLOSURES

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