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Controlled expression of cardiac-directed adenylylcyclase type VI provides increased contractile function

Mei Hua Gao[d], Hamed Bayat[a,b], David M. Roth[a,c], Jin Yao Zhou[d], Jeffrey Drumm[a], John Burhan[a,b], H. Kirk Hammond[a,b,*]

[a]*VA San Diego Healthcare System, 3350 La Jolla Village Drive, San Diego, CA 92161, USA*
[b]*Department of Medicine, University of California, San Diego, CA, USA*
[c]*Department of Anesthesiology, University of California, San Diego, CA, USA*
[d]*Collateral Therapeutics Incorporated, San Diego, CA, USA*

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Abstract

Objective: We have previously shown that cardiac-directed expression of adenylycyclase type VI (AC_{VI}) increases heart function in transgenic mice, and improves heart function and survival in murine cardiomyopathy. However, a potential problem of crossbreeding paradigms that use lines with two constitutively active transgenes is that results can be obfuscated by interactions between transgenes during growth and development. **Methods:** To develop a model that could be used subsequently to address this generic problem, transgenic mice with tetracycline (tet)-regulated cardiac-specific expression of AC_{VI} were generated. In this transgenic strain, the expression of a tet-controlled transactivator (tTA) was under control of the rat α-myosin heavy chain promoter. Expression of the AC_{VI} gene was driven by a tet-response element (TRE) and a minimal CMV promoter. **Results:** Homogenates of hearts showed no change in AC_{VI} protein content during tet suppression (doxycycline), confirming successful suppression of transgene expression. Removal of tet suppression for 10 days was associated with a 10-fold increase in cardiac AC_{VI} protein content. A similar increase in mRNA was observed (Northern blot analysis). The estimated half-life of newly synthesized cardiac AC_{VI} protein was 2–3 days. Isolated cardiac myocytes from animals that had tet-suppression removed for 10 days showed increased cAMP production in response to forskolin stimulation (Transgene Off: 15±6 fmol/μg; Transgene On: 39±14 fmol/μg; $n=5$ each group; $P=0.004$) and also to isoproterenol stimulation (Transgene Off: 20±5 fmol/μg; Transgene On: 31±12 fmol/μg; $n=5$ each group; $P=0.035$) and hearts isolated from these animals showed marked increased left ventricular peak dP/dt in response to dobutamine stimulation ($P=0.009$) indicating that inducible cardiac AC_{VI} is functionally coupled and recruitable. **Conclusion:** We have generated transgenic mice with controlled cardiac-specific expression of AC_{VI}, provided detailed information regarding the kinetics of transgene expression and suppression and estimated the half-life of cardiac AC_{VI} protein to be 2–3 days. Finally, we have shown, for the first time, that controlled cardiac-directed expression of a transgene can increase cardiac myocyte cAMP generation and left ventricular contractile function.

Keywords: Adrenergic (ant)agonists; Gender; Gene expression; Gene therapy; Heart failure; Myocytes

This article is referred to in the Editorial by P. Most et al. (pages 181–183) in this issue.

1. Introduction

A therapeutic transgene can be tested by exogenous gene delivery or by crossbreeding a transgenic line with a specific abnormality with another line expressing a potentially therapeutic gene. Crossbreeding paradigms circumvent difficulties associated with gene transfer and expression, providing useful information regarding potential therapeutic effects of specific genes. However, this widely used approach has several limitations [1]. For example, the expression of a therapeutic transgene during growth and

*Corresponding author. Tel.: +1-858-552-8585x3542; fax: +1-858-642-6213.
E-mail address: khammond@ucsd.edu (H. Kirk Hammond).

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development may prevent the index disease from ever developing in the first place. Furthermore, one can never be certain that a favorable outcome is not due to interactions between two transgenes that have little to do with a treatment effect per se. Preventing a disease state from ever manifesting is, admittedly, very different from treating a disease already present.

An approach with higher fidelity to clinical treatment of disease would be the transfer of a therapeutic gene when signs of heart failure, for example, are already present. Alternatively, this could be achieved by regulated expression of a therapeutic transgene. Exogenous control of transgene expression has been previously achieved in transgenic mice by using tetracycline (tet) transactivator or suppressor systems [2–5]. However, the utility of these systems to provide a regulated means to increase cardiac myocyte function per se has not been demonstrated.

Taking advantage of both the tight regulation provided by the tet-off system in transgenic mice and cardiac-specific expression provided by the α-myosin heavy chain (α-MHC) promoter, Yu et al. developed a transgenic mouse in which transgene expression was tissue-specific and regulatable [5]. They crossed transgenic mice harboring the tet-controlled transactivator (tTA) gene under the control of the α-MHC promoter (for cardiac-specific expression of tTA) with a transgenic line carrying a reporter gene under the control of the tTA-responsive promoter (for tet-inducibility). They demonstrated that in the double-transgenic offspring, the reporter gene was significantly induced in cardiac tissue after tetracycline withdrawal and displayed very little background expression in the presence of tetracycline. However, their study failed to prove that regulated expression of a cardiac transgene could alter cardiac function. Using a similar system to obtain regulated and cardiac-directed expression of a modified Gi-coupled receptor, Redfern et al. were able to induce cardiomyopathy [6]. However, no one has achieved a regulated means to increase cardiac function.

In the current study we have generated transgenic mice that express murine AC_{VI} in a cardiac-specific and regulatable manner. We show that AC_{VI} expression is inducible in the hearts of these animals, provide data regarding the kinetics of transgene expression and suppression, including the estimated half-life of newly synthesized cardiac AC_{VI} protein, and document the functional sequelae of regulated expression of AC_{VI} in cardiac myocytes and in isolated hearts.

2. Methods

We generated transgenic mice that expressed cardiac-directed AC_{VI} under tet regulation, established the cardiac specificity and kinetics of transgene expression and examined effects of regulated transgene expression on cardiac myocyte signaling. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication 85-23, revised 1996).

2.1. Generation of transgenic mice

To generate mice with cardiac-directed regulatable expression of AC_{VI}, a murine AC_{VI} cDNA was subcloned downstream of a minimal CMV promoter controlled by a tetracycline response element (TRE.CMV.min). TRE.CMV.min was excised from a pRetro-On vector (Clontech, Palo Alto, CA, USA) and both TRE.CMV.min and murine AC_{VI} cDNA were subcloned into pBluescript II SK vector (Stratagene, San Diego, CA, USA). A 7-kb fragment containing the expression cassette of the construct was used for microinjection. Pronuclear injection was carried out in the transgenic mouse facility at University of California, San Diego according to standard techniques into mouse ovum (strain C57B6/F1). Founder mice were identified by polymerase chain reaction (PCR) of genomic DNA prepared from tail tips. The tTA mice (strain B6/CBAF1, provided by Dr. G.I. Fishman) express a tetracycline-controlled transactivator (tTA) under the control of a 2.9-kb rat α-myosin heavy chain promoter [5]. The animals of line 6, aged 3–6 months, were used for this study.

2.2. Polymerase chain reaction (PCR) analysis

The AC_{VI} transgene was identified with primers from CMV.min promoter region (CMVP: 5′-GCAGAGCTCGTTTAGTGAAC-3′) and the AC_{VI} gene (AC_{VI} P1: 5′-CAGGAGGCCACTAAACCATGAC-3′) resulting in a 224-bp PCR product. The tTA transgene was identified using identical primers as described by Yu et al. [5]. To determine whether cardiac AC_V mRNA content was changed by increased cardiac AC_{VI} expression, reverse transcription and the polymerase chain reaction (RT-PCR) was used on samples of heart obtained from transgenic mice with constitutive cardiac-directed expression of AC_{VI} and their transgene negative siblings. The 3′-end anti-sense primer (5′-GTCAAAGCGGGCGAAGAGCTC) was located in exon 2 of the AC_V gene and used in reverse transcription. The 5′-end sense primer (5′-GAGGGCATCTGGTGGACCGTG) was located in exon 1 of the AC_V gene. The PCR result, using the 5′ and 3′ primers from AC_V cDNA was 526 bp. In the RT reaction, 5 μg of total RNA from each heart was used as template. The RT was performed using the SuperScript II kit and instructions from Invitrogen. The PCR reaction was performed as described above except that serial dilutions of the RT products were used as templates.

2.3. Documentation of transgene expression

Detection of AC_{VI} mRNA and protein were performed

as described previously [7]. Total RNA from samples of heart was extracted, separated on a 1.0% formaldehyde–agarose gel, and transferred onto a nylon membrane. After documenting equal RNA loading and successful RNA transfer (18S and 28S rRNA), AC_{VI} mRNA was identified with a [^{32}P]dCTP-labeled murine AC_{VI} cDNA probe. Endogenous vs. transgene levels of AC_{VI} could be independently evaluated because transgene AC_{VI} was of different mobility due to the presence of partial 5′- and 3′-untranslated regions. To detect AC_{VI} protein, a polyclonal antibody recognizing AC_V and AC_{VI} proteins (Santa Cruz Biosciences) was used in immunoblotting conducted on cardiac homogenates. Total cardiac membrane protein (100 μg) was separated on 7.0% PAGE and transferred to a nitrocellulose membrane. The AC_{VI} protein was detected by incubating the membrane with anti-AC_V/AC_{VI} primary antibody followed by a goat anti-rabbit IgG horseradish peroxidase conjugate (Gibco-BRL Life Technology).

2.4. Regulated expression of transgene

To repress tTA-dependent transactivation, the water supply included doxycycline (0.2 mg/ml) and 2% sucrose. To induce the expression of transgene AC_{VI}, doxycycline was withdrawn from the water and animals killed 10 days later. Doxycycline inhibits matrix metalloproteinases (MMP), which can be associated with alterations in left ventricular geometry [8]. We found no adverse effects of doxycycline on cardiac structure or function when administered to mice for as long as 15 months.

2.5. Kinetics of AC_{VI} protein expression

The kinetics of induced expression of AC_{VI} was studied by removing doxycycline from drinking water for 2, 4, 6, 8, 10, 12 and 14 days. Hearts (one mouse per time point) were obtained at each time point and divided into equal portions to determine mRNA (Northern) and protein expression (immunoblotting).

2.6. Reversal of transgene expression and time course of AC_{VI} protein

Transcription of AC_{VI} mRNA, and therefore protein expression, once induced, can be rapidly suppressed again by adding doxycycline to the drinking water. This provided an opportunity to determine how long newly synthesized cardiac AC_{VI} mRNA and protein would endure. This was achieved by Northern and Western analysis. Half of the heart from each mouse was used for mRNA analysis (Northern blotting) the remainder for protein detection (immunoblotting). AC_{VI} gene expression was induced in the animals by removing doxycycline from the drinking water, and subsequently suppressed by adding doxycycline back for 1, 2 or 3 days. In these studies, we set the initially induced cardiac AC_{VI} protein content to be 100% and then examined the amount remaining 1, 2 and 3 days after suppression, thereby obtaining an indirect assessment of how long newly synthesized cardiac AC_{VI} mRNA and protein endures.

2.7. Cardiac myocyte cAMP production

Cyclic AMP (cAMP) production in the heart of transgenic mice was measured from isolated cardiac myocytes as described previously [7]. Cardiac myocytes were isolated after intracoronary perfusion and digestion of the heart with perfusion medium (Joklik-modified minimum essential medium with 10 mM Na-HEPES, 30 mM taurine, 2 mM carnitine, and 2 mM creatine; pH 7.36) and collagenase (Worthington Type II, 358 I.U./mg, 1 mg/ml). Isolated cardiac myocytes were stimulated (10 min) with forskolin (10 μM) or isoproterenol (10 μM). Cyclic AMP was extracted from cells using 7.5% ice-cold trichloroacetic acid and measured by radioimmunoassay (Amersham Life Science).

2.8. Left ventricular contractile function

Cardiac function in response to adrenergic stimulation was assessed in isolated perfused hearts (LV end-diastolic pressure 10 mmHg; 1.7 mM ionized Ca^{2+}) using an intraventricular balloon catheter to measure isovolumic LV pressure as previously described [9]. Dobutamine (0.1, 1 and 10 μM) was delivered in bolus doses at 5-min intervals as LV pressure was recorded. Data were collected and analyzed blinded to group identity.

2.9. Statistical analysis

Data are reported as mean±1 standard error of the mean. Group comparisons were made using repeated measures analysis of variance (physiological data at multiple concentrations) or Student's *t* test (two-tailed) (biochemical parameters when testing between two group means).

3. Results

3.1. Generation of transgenic mice

Six founders were identified from forty-two live births resulting from pronuclear injection with the CMV.min.AC_{VI} DNA fragment. These mice were crossbred with 2.9αtTA mice (Fig. 1) and offspring were screened for both tTA and AC_{VI} genes by PCR.

3.2. Regulated expression of transgene

The double-positive mice (containing both tTA and

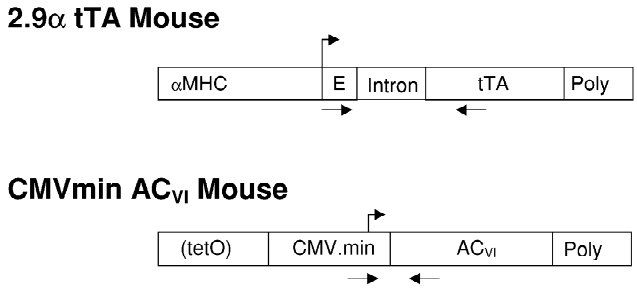

Fig. 1. Transgenic mouse lines used to generate lines with cardiac-directed controlled expression of AC_{VI}. The arrows below each diagram indicate PCR primers.

AC_{VI} genes) resulting from mating tTA and AC_{VI} transgenic lines were divided into two groups, one receiving doxycycline (Transgene Off), and the other not receiving doxycycline (Transgene On). The expression level of AC_{VI} protein was then compared in hearts obtained from animals in the two groups. Double-negative siblings (lacking tTA and AC_{VI}) were used to assess the endogenous level of cardiac AC_{VI} protein, which was, as expected, quite low. Withdrawal of doxycycline from the drinking water resulted in >5-fold increase in AC_{VI} mRNA and a 10-fold increase of cardiac AC_{VI} protein (Fig. 2) in mice from line 6; mice from line 5 also showed increased AC_{VI} mRNA and protein after doxycycline withdrawal—although to a somewhat lesser degree. However, the remaining four lines did not show increased expression of cardiac AC_{VI} mRNA or protein over background levels (See line 4, Fig. 2B). The suppression of AC_{VI} protein expression by doxycycline was effective since cardiac protein content was indistinguishable from double-negative control mice (Fig. 2).

These results suggest that the response of TRE.CMV.min promoter to doxycycline is dependent on the integration site of the transgene. The tight suppression achieved by doxycycline on AC_{VI} expression indicates that the chance of leakage of transgene expression from TRE.CMV.min promoter is small. Once an inducible line is established the transgene is inherited from generation to generation in a stable manner. Line 6 has been bred for seven generations, and both the inducibility and robustness of the expression of the transgene have not changed (data not shown).

3.3. Kinetics of AC_{VI} expression

Following the removal of doxycycline from the water, cardiac AC_{VI} mRNA and protein were increased by day 4 and reached a plateau 10 days later (Fig. 3), but because of limited sample size at each time point, we emphasize that these are estimates.

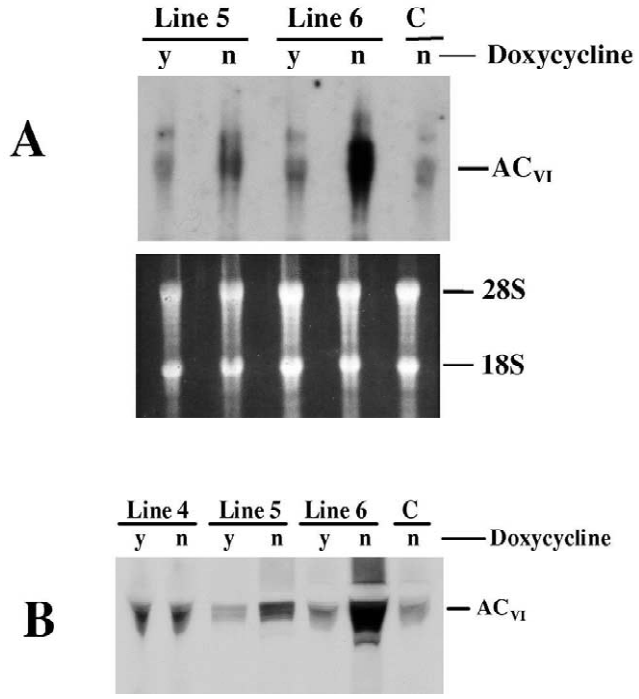

Fig. 2. Northern (A) and Western (B) analysis of regulated cardiac AC_{VI} expression. In both panels, mice receiving doxycycline continuously are denoted by y; mice that had doxycycline removed from water for 10 days are denoted by n. Therefore, n indicates no transgene suppression and y denotes continuous transgene suppression. (A) Detection of transgene AC_{VI} message using a [^{32}P]dCTP-labeled AC_{VI} DNA probe. Data from heart samples from two lines of mice (lines 5 and 6) and a double transgene negative sibling Control mouse (C) are shown. The data confirm a marked increase in cardiac AC_{VI} mRNA content associated with removal of doxycycline from the water supply. RNA loading was assessed by ethidium bromide staining of the 1.0% formaldehyde–agarose gel showing equivalent 18S and 28S bands. (B) Detection of AC_{VI} protein by Western blotting. Data from heart samples from three lines of mice (lines 4–6) and a double transgene negative sibling Control mouse (C) are shown. Hearts from line 4 animals did not respond to doxycycline. Hearts from lines 5 and 6 animals show increased cardiac AC_{VI} protein content associated with removal of doxycycline from the water supply.

3.4. Reversal of transgene expression and time course of AC_{VI} protein

We next asked how long after reaching a plateau of mRNA and protein expression would transgene expression still be detectable after reinstituting transgene suppression with doxycycline—an experiment that would provide data regarding the half-life of newly synthesized AC_{VI}. Transgene AC_{VI} mRNA in hearts from mice receiving doxycycline for 1 day was reduced to endogenous levels—transcription of transgene AC_{VI} mRNA was completely suppressed 1 day after doxycycline addition (Fig. 4). Of newly synthesized cardiac AC_{VI} protein, the amount remaining 1, 2 and 3 days after reinstitution of suppression was 96, 71 and 38%, respectively (Fig. 4). These data

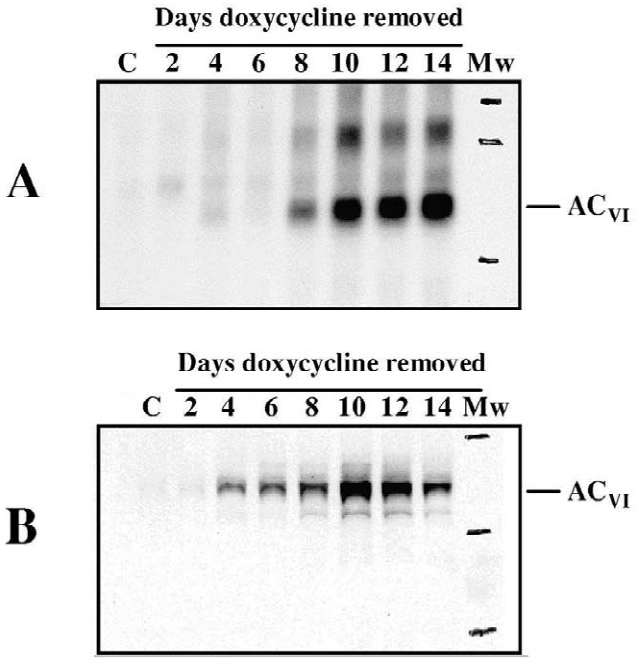

Fig. 3. Kinetics of regulated cardiac AC_{VI} expression. Control mice (C) are transgene negative siblings that received doxycycline continuously. Other lanes represent transgene positive animals that had doxycycline removed from water for 2–14 days. (A) Northern blot analysis. A 20-μg amount of total RNA from sample hearts was loaded per lane. Detection of cardiac transgene AC_{VI} mRNA was evident 4 days after removal of doxycycline, but did not reach an apparent maximal amount until 10 days after removal. (B) Western blot analysis. A 50-μg amount of protein from sample hearts was loaded per lane. Detection of increased cardiac AC_{VI} protein was evident 4 days after removal of doxycycline, but did not reach an apparent maximal amount until 10 days after removal.

provide an estimated half-life of newly synthesized AC_{VI} of 2–3 days. We found that in the constitutively expressed cardiac-directed AC_{VI} transgenic mouse, expression of cardiac AC_V appears to be unchanged (Fig. 5A).

3.5. Tissue-specific regulation and expression

Transcription of transgene AC_{VI} was restricted to the heart. Northern blot analysis showed that there was no expression of transgene AC_{VI} message in brain, kidney, liver, lung, skeletal muscle and spleen (Fig. 5B). These data indicate that cardiac-directed expression of tTA and lack of leakage of AC_{VI} expression from TRE.CMV.min promoter provides cardiac-specific expression.

3.6. Functional assessment of regulated transgene

To determine whether controlled expression of cardiac-directed AC_{VI} could lead to important functional consequences, we measured cAMP production in isolated cardiac myocytes and left ventricular pressure development in isolated perfused hearts. Regulated expression of cardiac-directed AC_{VI} resulted in increased cAMP production in response to forskolin (Transgene Off: 15±6 fmol/μg; Transgene On: 39±14 fmol/μg; $P=0.0044$; $n=5$ per group), and isoproterenol (Transgene Off: 20±5 fmol/μg; Transgene On: 31±12 fmol/μg; $P=0.035$; $n=5$ per group; Fig. 6). Basal cAMP levels were unchanged, as previously reported in examples in which AC_{VI} was expressed in normal heart or in normal cardiac myocytes [7,10]. We have previously reported that increasing the amounts of cardiac AC_{VI} is not associated with alterations in myocardial β-adrenergic receptor number or in the amounts of Giα2 or Gsα [7,9].

Hearts isolated from the two groups showed similar basal heart rates (Transgene Off: 231±26 bpm, $n=4$; Transgene On: 245±17 bpm, $n=4$; $P=0.67$) and basal LV dP/dt (Fig. 6; $P=0.31$). However, hearts isolated from animals that had transgene suppression removed showed marked increases in left ventricular dP/dt in response to dobutamine infusion ($P=0.009$; $n=4$ for each group; Fig. 6). These data indicate that regulated expression of cardiac AC_{VI} is functionally important, coupled and recruitable through β-adrenergic receptor stimulation.

4. Discussion

We have generated a transgenic mouse model with a cardiac-specific and regulatable AC_{VI} transgene expression. AC_{VI} transgene protein content was indistinguishable from normal mice when suppressed, increased 10-fold when activated, was rapidly reversed when suppression was reapplied, and expression was limited to the heart.

Of the six transgenic lines that were positive for the transgene, two lines showed transgene expression upon activation, while four lines did not. This is likely due to the integration site of the transgene expression cassette within the chromosome [11]. The lack of expression in four of the lines may be due to the integration of the transgene expression cassette in an area subjected to a silencer effect [12]. Thus, due to the variability observed between transgenic lines, screening of a high number of transgenic animals may be required to obtain an adequate induction of the transgene. On the other hand, once an inducible line was obtained, the transgene cassette was efficiently transmitted from generation to generation (so far, up to seven generations), showing that the transgenic line was stable over time.

In the tet-off transgenic line generated in this study, AC_{VI} protein levels were induced 10-fold over endogenous AC_{VI} levels 10 days after removal of doxycycline. Isolated cardiac myocytes from these animals showed increased cAMP production in response to isoproterenol and forskolin stimulation and their hearts showed marked increases in left ventricular pressure development during β-adrenergic receptor stimulation. We have previously

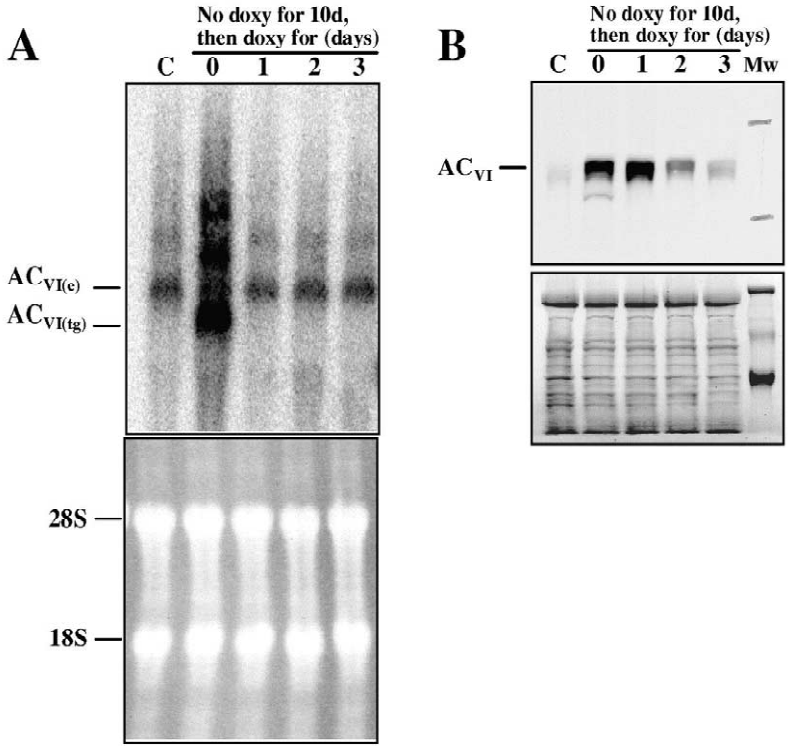

Fig. 4. Increased cardiac AC_{VI} transgene expression is reversible. Control mice (C) are transgene negative siblings that received doxycycline continuously. Other lanes represent transgene positive animals that had doxycycline removed from water 10 days, thereby removing inhibition of cardiac AC_{VI} transgene expression, followed by reinstitution of doxycycline for 0–3 days to determine the kinetics of transgene suppression. Doxy, doxycycline; d, days; $AC_{VI(e)}$, endogenous cardiac AC_{VI} mRNA; $AC_{VI(tg)}$, transgene cardiac AC_{VI} mRNA (lower due to partial 5′- and 3′-untranslated regions). (A) Cardiac transgene AC_{VI} mRNA was detected using a [^{32}P]dCTP-labeled AC_{VI} DNA probe in Northern blot analysis. Transgene AC_{VI} mRNA expression was markedly decreased 1 day after reinstitution of doxycycline. Equivalent RNA loading was confirmed by examination of 18S and 28S band size. (B) Cardiac transgene AC_{VI} protein. Cardiac homogenates from the same mice shown in (A) were used for AC_{VI} protein detection using immunoblotting. Transgene AC_{VI} protein expression was markedly decreased 2–3 days after reinstitution of doxycycline. Equal amounts of membrane protein were loaded into each lane as shown in the lower panel (Ponceau staining).

demonstrated that, in transgenic mice expressing AC_{VI} in a cardiac-specific, constitutive manner, 20-fold overexpression of AC_{VI} resulted in a similar increase in cAMP production and an enhanced responsiveness of the heart to stress [7]. Furthermore, in a somatic gene transfer study, we showed that cardiac responsiveness is increased even when cardiac AC_{VI} is increased 2-fold [13]. Thus, the magnitude of induction observed in our cardiac-specific, tet-off AC_{VI} transgenic mice should be sufficient to achieve a therapeutic effect in the setting of heart failure.

We also demonstrated the reversibility of AC_{VI} induction: newly synthesized cardiac AC_{VI} protein was substantially reduced 3 days after the addition of doxycycline. Activation of transgene expression combined with rapid suppression is an advantageous characteristic of the regulation system that would be useful to test the therapeutic effect of AC_{VI} transgene in a heart failure model. If the transgene proves to have a therapeutic effect on heart failure, then turning-off transgene expression should result in a return of signs of heart failure.

Because of inability of available antibodies specific for AC_{VI} to precipitate the protein, pulse labeling studies have not been possible. As a result, there are no data regarding the putative biological half-life of AC_{VI}. Generation of mice with cardiac-directed regulatable expression of AC_{VI} provided an opportunity to assess the biological half-life of AC_{VI}. Taking advantage of the fact that we could rapidly and completely suppress the expression of transgene AC_{VI}, we showed that newly synthesized AC_{VI} has an approximate half-life in the heart of 2–3 days.

The transgenic construct was generated such that the expression of the transactivator (tTA), which provides tet-regulated expression, is under the control of the cardiac-specific αMHC promoter. This feature should allow constitutive expression of tTA in the heart only. In the heart, tTA should interact with the tet operon placed upstream the AC_{VI} transgene, and thereby provide a tet-regulated, cardiac-specific expression of the transgene. We demonstrated that, indeed, AC_{VI} transgene expression was restricted to the heart tissues, as no transgene expression was detected in other tissues. In another study using a similar transgenic construct, transgene (luciferase) expression was also induced in the lungs [5]. The lack of specificity of the αMHC promoter could explain this

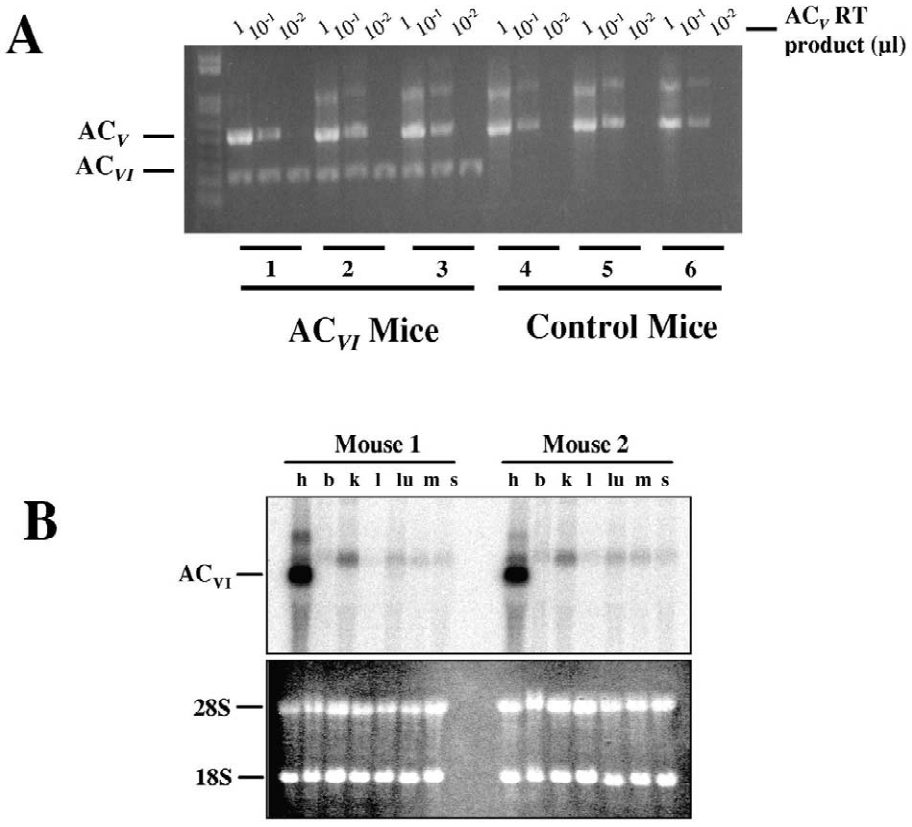

Fig. 5. (A) AC_V expression is not altered by cardiac-directed expression of AC_{VI}. Hearts were obtained from transgenic mice with cardiac-directed expression of AC_{VI} (mice 1–3) and in transgene negative siblings (Control Mice) from the same line (mice 4–6). RT-PCR shows no increase in mRNA levels for AC_V. (B) Heart-specific expression of cardiac-directed transgene AC_{VI}. Twenty µg of total RNA from several tissues was used for Northern blot analysis. Studies were conducted on tissues obtained from two transgene positive siblings (Mouse 1 and Mouse 2) after removal of doxycycline. Transgene expression was easily detectable in heart, but was undetectable in other organs. Equivalent RNA loading was confirmed by 18S and 28S band size. h, heart; b, brain; k, kidney; l, liver; lu, lung; m, skeletal muscle; s, spleen.

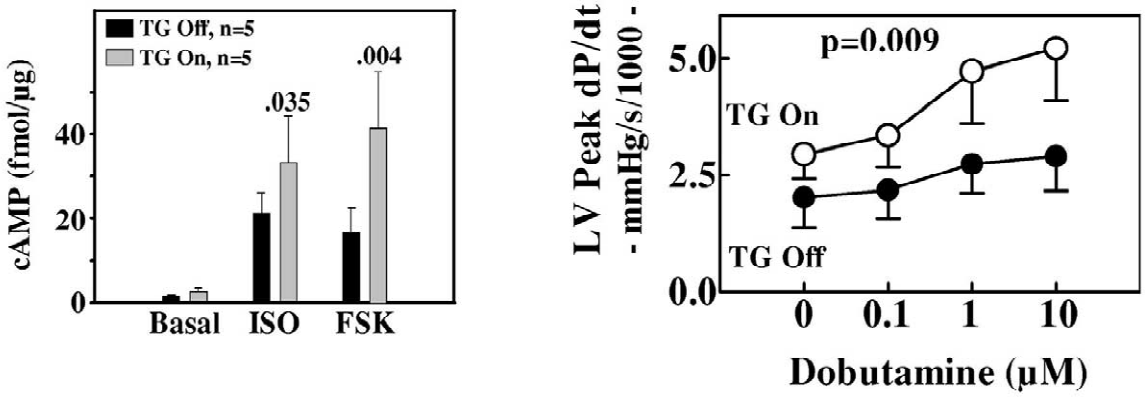

Fig. 6. Left panel: β-adrenergic responsiveness and AC function in cardiac myocytes. To determine whether controlled expression of cardiac-directed AC_{VI} could lead to important functional consequences, we measured cAMP production in isolated cardiac myocytes. Cardiac myocytes were isolated from ten animals—five that had received doxycycline continuously (TG Off) and another five that had doxycycline withdrawn for 10 days (TG On). Isolated cardiac myocytes were stimulated with 10 µM isoproterenol (ISO) and 10 µM forskolin (FSK). These data indicate that regulated expression of cardiac AC_{VI} is functionally important, coupled and recruitable through β-adrenergic receptor stimulation of isolated cardiac myocytes. Bars represent mean values; error bars denote 1 S.D. Number above bars is *P* value (TG On vs. TG Off). Right panel: β-adrenergic stimulation of isolated hearts. To determine whether controlled expression of cardiac-directed AC_{VI} was associated with alterations in left ventricular function, hearts were isolated from eight animals—four that had received doxycycline continuously to suppress transgene AC_{VI} expression (TG Off, closed circles) and another four that had doxycycline withdrawn for 10 days (TG On, open circles). These hearts were then perfused with graded doses of dobutamine as left ventricular peak pressure development (LV d*P*/d*t*), a measure of contractile function, was measured. These data indicate that regulated expression of cardiac AC_{VI} is functionally important, coupled and recruitable. Bars represent mean values; error bars denote 1 S.E.M.

observation; indeed, the αMHC promoter has been shown to yield low level of expression in noncardiac tissues in a transgenic setting [14].

An important limitation to standard crossbreeding paradigms examining the effects of potential therapeutic transgenes in treating genetic models of heart failure is that the therapeutic transgene is present prior to the development of heart failure. This model will facilitate experiments in which heart failure is fully developed before AC_{VI} is activated — thereby providing a more stringent test than crossbreeding paradigms. In addition, the model enables a thorough study of cardiac AC signaling in heart failure.

Perhaps a few words regarding the overall strategy of using AC as a potential treatment for heart failure are appropriate, even though therapy per se is not addressed in the current experiments. Agents that increase intracellular levels of cAMP have been used to treat clinical heart failure but results of these clinical trials have been disappointing, perhaps because the agents used (β-adrenergic receptor agonists, milrinone) provided sustained increases of intracellular cAMP. In contrast, sustained increases in cAMP are not observed in cardiac myocytes expressing AC_{VI} [7,9,10,13,15]. We have recently shown that when this strategy is applied to a genetic model of dilated cardiomyopathy, survival and LV function are markedly improved [15]—in contrast, when the same cardiomyopathy model is treated with the overexpression of β-adrenergic receptors, life is shortened [16], underscoring a key difference between receptor- and effector-targeted gene transfer with regard to β-adrenergic receptor signaling.

In conclusion, we have generated transgenic mice with controlled cardiac-specific expression of AC_{VI}. In addition, we have provided detailed information regarding the kinetics of transgene expression and suppression, and have estimated the half-life of cardiac AC_{VI} protein to be 2–3 days. Finally, we have shown, for the first time, that controlled cardiac-directed expression of a transgene can increase cardiac myocyte cAMP generation and left ventricular contractile function.

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