Cardiac βARK1 inhibition prolongs survival and augments β blocker therapy in a mouse model of severe heart failure

Victoria B. Harding*, Larry R. Jones†, Robert J. Lefkowitz*‡, Walter J. Koch§, and Howard A. Rockman*¶

Departments of *Medicine and §Surgery, and ‡Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710; and †Krannert Institute of Cardiology and Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

Contributed by Robert J. Lefkowitz, March 1, 2001

Chronic human heart failure is characterized by abnormalities in β-adrenergic receptor (βAR) signaling, including increased levels of βAR kinase 1 (βARK1), which seems critical to the pathogenesis of the disease. To determine whether inhibition of βARK1 is sufficient to rescue a model of severe heart failure, we mated transgenic mice overexpressing a peptide inhibitor of βARK1 (βARKct) with transgenic mice overexpressing the sarcoplasmic reticulum Ca^{2+}-binding protein, calsequestrin (CSQ). CSQ mice have a severe cardiomyopathy and markedly shortened survival (9 ± 1 weeks). In contrast, CSQ/βARKct mice exhibited a significant increase in mean survival age (15 ± 1 weeks; $P < 0.0001$) and showed less cardiac dilation, and cardiac function was significantly improved (CSQ vs. CSQ/βARKct, left ventricular end diastolic dimension 5.60 ± 0.17 mm vs. 4.19 ± 0.09 mm, $P < 0.005$; % fractional shortening, 15 ± 2 vs. 36 ± 2, $P < 0.005$). The enhancement of the survival rate in CSQ/βARKct mice was substantially potentiated by chronic treatment with the βAR antagonist metoprolol (CSQ/βARKct nontreated vs. CSQ/βARKct metoprolol treated, 15 ± 1 weeks vs. 25 ± 2 weeks, $P < 0.0001$). Thus, overexpression of the βARKct resulted in a marked prolongation in survival and improved cardiac function in a mouse model of severe cardiomyopathy that can be potentiated with β-blocker therapy. These data demonstrate a significant synergy between an established heart-failure treatment and the strategy of βARK1 inhibition.

The β-adrenergic receptor (βAR) signaling pathway is one of the key pathways regulating cardiac function. However, chronic stimulation of βARs, which occurs in heart failure, leads to chronic desensitization and impaired βAR responsiveness. This process of agonist-induced βAR desensitization requires phosphorylation of the agonist-occupied receptor by the cytosolic enzyme βAR kinase 1 (βARK1), which is recruited to the plasma membrane through its interaction with dissociated membrane-bound Gβγ-subunits (1).

Although the molecular mechanisms involved in the pathological progression to decompensated heart failure are not well understood, a leading candidate is impaired βAR signaling. Abnormalities in βAR signaling that characterize human heart failure include a 50% reduction in βAR density selectively involving the β_1AR subtype, marked uncoupling of remaining β_1 and β_2ARs, and an ≈3-fold increase in βARK1 levels and activity (2, 3).

Whether down-regulation and desensitization of βAR function are adaptive or maladaptive in the failing heart remains controversial. In this regard, an important role of βARK1 in the pathogenesis of heart failure was recently demonstrated in a mouse model of cardiomyopathy wherein mice overexpressing a cardiac-targeted peptide inhibitor of βARK were significantly protected from the development of myocardial failure (4). To inhibit the βARK–Gβγ interaction, a strategy of Gβγ sequestration was achieved by overexpression of the C-terminal 194 aa of βARK1 (βARKct), which effectively inhibits the action of βARK1 and augments βAR responsiveness (5). In a similar approach, βARKct expression through adenoviral gene delivery in rabbit hearts at the time of myocardial infarction significantly delayed the development of heart failure (6). Thus, βARKct expression and subsequent βARK1 inhibition seem to positively affect the failing heart.

Because sudden cardiac death is a prominent feature of the clinical syndrome of human heart failure, we wanted to test whether normalizing βAR function through βARK inhibition would improve survival. To test this possibility, we used a model of severe heart failure generated by cardiac overexpression of the sarcoplasmic reticulum Ca^{2+}-binding protein calsequestrin (CSQ); this model is characterized by an aggressive phenotype of dilated cardiomyopathy and premature death by 16 weeks of age (7, 8). In addition to using a βARKct transgenic mouse crossbreeding strategy to attempt to rescue CSQ heart-failure mice, we tested how βARK1 inhibition compared with chronic βAR-antagonist treatment—a standard therapy for heart failure—and whether βARK1 inhibition could provide additional benefit to β-blocker therapy.

Methods

Experimental Animals. Transgenic mice overexpressing either CSQ or the βARKct peptide were generated as described (5, 7). Briefly, full-length canine cardiac CSQ cDNA was fused to the α-myosin heavy-chain promoter to drive cardiac-targeted expression. For the βARKct, the coding sequence for the last 194 aa of bovine βARK1 was fused to the α-myosin heavy-chain promoter. F_1 pups were generated from the crossbreeding of CSQ transgenic mice with βARKct transgenic mice. Wild-type, CSQ transgenic, βARKct transgenic, or CSQ/βARKct hybrid transgenic littermates of either sex were used for all studies.

In separate experiments, mice were chronically treated with the selective β_1AR antagonist metoprolol (350 mg/kg of body weight per day) administered in the drinking water (2 mg/ml) starting from 1 week of age and continuing until death. The animals in this study were handled according to approved protocols and the animal welfare regulations at Duke University.

Transthoracic Echocardiography. Serial echocardiography was performed on conscious mice with an HDI 5000 echocardiograph (ATL Ultrasound, Bothell, WA) at 7 and 11 weeks of age, as described (9).

Cardiac Catheterization. Hemodynamic evaluation in intact closed-chest anesthetized mice was performed as described (4).

MEDICAL SCIENCES

Abbreviations: βAR, β-adrenergic receptor; βARK1, βAR kinase 1; βARKct, C-terminal of βARK1; CSQ, calsequestrin; LV, left ventricular; MLP, muscle LIM protein.

¶To whom reprint requests should be addressed at: Department of Medicine, Duke University Medical Center, DUMC 3104, Durham, NC 27710. E-mail: h.rockman@duke.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

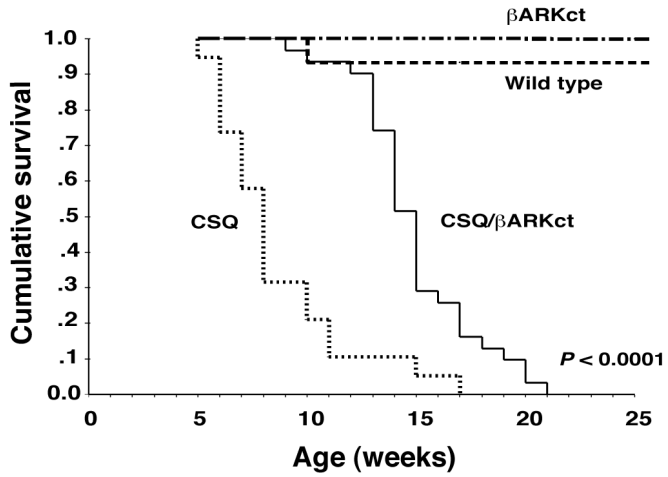

Fig. 1. Survival analysis in CSQ/βARKct mice and CSQ mice. Kaplan–Meier survival analysis was used to determine the survival probability between the different genotypes of mice from the CSQ-βARKct cross. Mean survival age of the CSQ mice was 9 ± 1 weeks vs. 15 ± 1 weeks in the CSQ/βARKct mice ($P <$ 0.0001). Wild type, $n = 15$; βARKct, $n = 23$; CSQ, $n = 14$; CSQ/βARKct, $n = 31$.

Membrane Preparation and Immunoblotting. Briefly, left ventricles were homogenized and sarcolemmal membranes were prepared as described (5, 10). Cytosolic extracts were then used for immunoblotting; membranes were used in the adenylyl cyclase, βAR density, and βAR competition binding assays. For protein expression, 100 μg (βARK1 and βARKct) or 10 μg (CSQ) of cytosolic extracts was immunoblotted by using rabbit polyclonal antibodies raised against either an epitope on the C terminus of GRK2 (1:500) (Santa Cruz Biotechnology) or CSQ (1:2000) (L.R.J.), βARK1 protein levels were quantified by densitometry.

Adenylyl Cyclase Activity, βAR Density, and Radioligand Binding. For cyclase activity, membranes (15 μg of protein) were incubated for 20 min at 37°C with [α-^{32}P]ATP under basal conditions or indicated agonists and cAMP was quantified (5, 10).

Competition binding isotherms in sarcolemmal membranes (25 μg) were done in triplicate with 12 concentrations of isoproterenol (10^{-10} M to 10^{-4} M). Assays were performed at 37°C for 60 min (5, 10). Competition isotherms were analyzed by nonlinear least-square curve fit to determine the percentage of βARs in a high-affinity state (GraphPad PRISM).

Statistical Analysis. Data are expressed as mean ± SE. Survival data were analyzed by using a Kaplan–Meier survival analysis with a log rank method of statistics. Statistical significance for echocardiographic variables was performed with a one-way ANOVA for 7-week data and a repeated-measures ANOVA for the serial echocardiographs. βARK1 levels were compared with an unpaired Student's t test.

Results

To determine whether normalizing βAR function by inhibiting the βARK–Gβγ interaction would improve survival in heart failure, we crossed transgenic mice with cardiac-targeted overexpression of the βARKct with transgenic mice overexpressing CSQ. Single transgenic CSQ mice and binary transgenic CSQ/βARKct mice were monitored for survival and compared with their wild-type littermates. Whereas the CSQ mice had a mean survival of only 9 ± 1 weeks, the CSQ/βARKct mice showed a significant increase in lifespan, with a mean survival age of 15 ± 1 weeks ($P < 0.0001$; Fig. 1). This result demonstrates that βARK1 inhibition has a significant positive effect on survival.

To determine whether overexpression of the βARKct would affect the dilated cardiomyopathy phenotype in the CSQ mice, transthoracic echocardiography was performed in conscious mice at 7 weeks of age. Compared with wild-type and single βARKct transgenic mice, CSQ mice have enlarged cardiac chambers, as shown by the increased left ventricular (LV) end-diastolic and end-systolic dimensions, and severe cardiac dysfunction, as shown by the markedly reduced fractional shortening and mean velocity of circumferential fiber shortening (mVcfc) (Table 1 and Fig. 2 *A* and *B*). In contrast, the CSQ/βARKct mice had significantly less cardiac dilation (25%, $P <$ 0.0001) and significantly improved cardiac function (2-fold, $P <$ 0.0001), as compared with their CSQ littermates.

To further evaluate the effects of βARKct expression on the progression of cardiac failure in CSQ mice, serial echocardiography was performed in the surviving transgenic and wild-type mice. Individual data points are plotted for LV end diastolic dimension and fractional shortening from echocardiograms recorded at 7 and 11 weeks. Cardiac function in CSQ mice was severely depressed at 7 weeks and did not significantly change from 7 to 11 weeks (Fig. 2 *C* and *D*); however, most CSQ mice

Table 1. Echocardiographic and physiologic parameters at 7 weeks of age

Parameter	Wild type $n = 15$	βARKct $n = 23$	CSQ $n = 14$	CSQ/βARKct $n = 31$	CSQ (metoprolol) $n = 18$	CSQ/βARKct (metoprolol) $n = 18$
LVEDD, mm	2.98 ± 0.10	3.26 ± 0.08	5.60 ± 0.17*	4.19 ± 0.09†§	5.15 ± 0.14¶	4.01 ± 0.12‖
LVESD, mm	1.02 ± 0.07	1.26 ± 0.07	4.79 ± 0.25*	2.71 ± 0.11†§	4.03 ± 0.18¶	2.46 ± 0.15‖
FS, %	66 ± 2	61 ± 1	15 ± 2*	36 ± 2†§	22 ± 2¶	39 ± 2‖
SEPth, mm	0.78 ± 0.04	0.76 ± 0.02	0.55 ± 0.02*	0.75 ± 0.03†§	0.63 ± 0.03¶	0.70 ± 0.03
PWth, mm	0.80 ± 0.06	0.76 ± 0.03	0.59 ± 0.03*	0.79 ± 0.03†§	0.62 ± 0.02	0.73 ± 0.02‖
HR, beats/min	565 ± 11	588 ± 16	579 ± 33	564 ± 14	525 ± 8	527 ± 16
mVcfc, circ/sec	4.83 ± 0.16	4.68 ± 0.16	1.6 ± 0.18*	3.02 ± 0.16†§	1.79 ± 0.16¶	3.51 ± 0.24‖
dP/dt_{max} basal	10,054 ± 824	10,239 ± 722	3,443 ± 404*	4,812 ± 347‡		
dP/dt_{max} iso	17,973 ± 712	20,074 ± 892	4,641 ± 589*	6,261 ± 345‡		
LVW/BW, mg/g	3.53 ± 0.06	3.32 ± 0.05	8.53 ± 0.35*	6.20 ± 0.14†§	7.95 ± 0.15	6.75 ± 0.33‖
(*n*)	(16)	(11)	(11)	(20)	(17)	(14)

Analysis of *in vivo* cardiac size and function by echocardiography in gene-targeted mice. LVEDD, LV end diastolic dimension; LVESD, LV end systolic dimension; FS, fractional shortening; SEPth, septal wall thickness; PWth, posterior wall thickness; HR, heart rate; mVcfc, heart rate corrected mean velocity of circumferential fiber shortening; dP/dt_{max}, first derivative of LV pressure; LVW, LV weight; BW, body weight. (*n*), at bottom of table, the number of animals used for the hemodynamic study and calculation of LVW/BW. *, $P < 0.005$, CSQ vs. wild type; †, $P < 0.005$; ‡, $P < 0.05$ CSQ/βARKct vs. CSQ; §, $P < 0.01$, CSQ/βARKct vs. CSQ metoprolol; ¶, $P < 0.05$, CSQ metoprolol vs. CSQ; ‖, $P < 0.005$, CSQ/βARKct metoprolol vs. CSQ metoprolol.

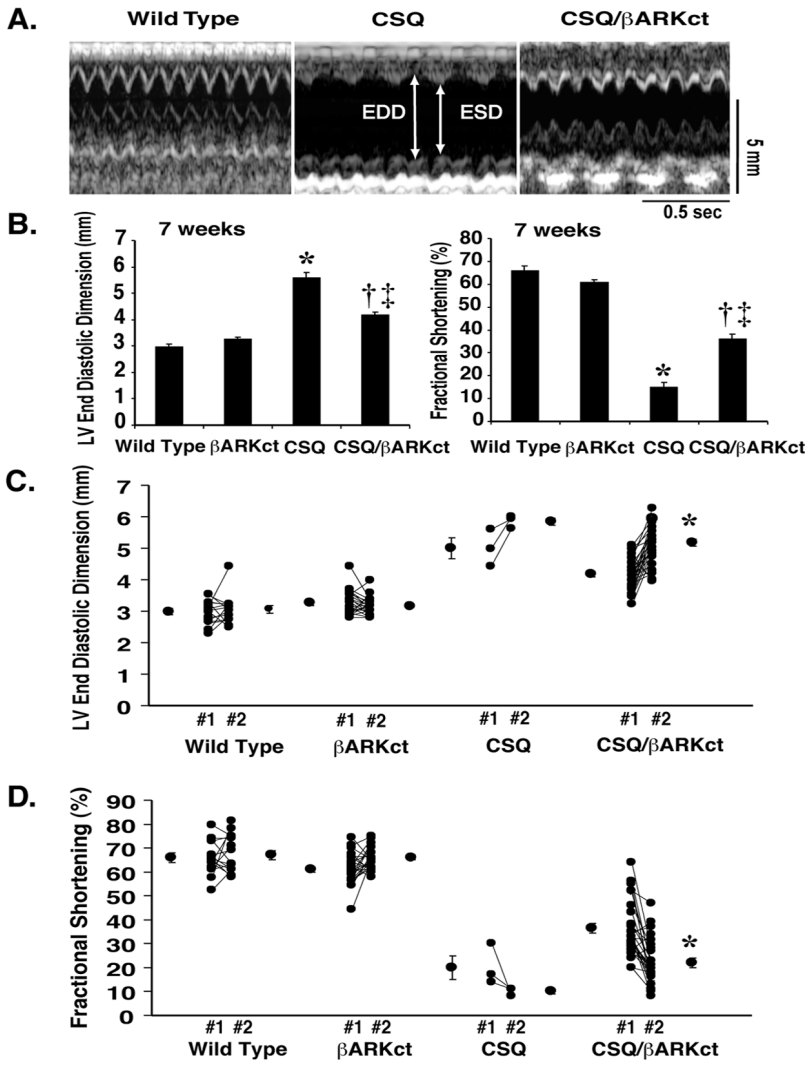

Fig. 2. Analysis of cardiac function by noninvasive echocardiography in conscious mice. (*A*) Transthoracic M-mode echocardiographic tracings in 7-week-old wild-type (*Left*), CSQ (*Center*), and CSQ/βARKct (*Right*) mice. LV dimensions are indicated with the double-sided arrows. EDD, end diastolic dimension; ESD, end systolic dimension. Wild-type mice have normal chamber size, whereas the CSQ mice have chamber dilation and depressed cardiac function. The CSQ/βARKct mice have only moderate chamber dilation and slightly reduced cardiac function as compared with the wild-type mice. (*B*) Echocardiographic findings in 7-week-old wild-type and transgenic mice. LV EDD (*Left*) and percent fractional shortening (*Right*) are shown. Wild type, $n = 15$; βARKct, $n = 23$; CSQ, $n = 14$; CSQ/βARKct, $n = 31$. *, $P < 0.0001$, CSQ vs. wild type; †, $P < 0.0001$, CSQ/βARKct vs. CSQ; ‡, $P < 0.0001$, CSQ/βARKct vs. wild type. (*C*) Data from serial echocardiograms in the same mouse at 7 weeks (#1) and 11 weeks (#2) of age for LV EDD. (*D*) Percent fractional shortening in the same mouse. Wild type, $n = 14$; βARKct, $n = 22$; CSQ, $n = 3$; CSQ/βARKct, $n = 28$. *, $P < 0.001$, CSQ/βARKct (#2) vs. CSQ/βARKct (#1).

did not survive to the 11-week time point because of the already severe stage of myocardial failure in the mice at 7 weeks of age. In contrast, cardiac function in the CSQ/βARKct mice at 7 weeks of age was significantly improved in comparison to CSQ mice, and although there was a decline in cardiac function from 7 to 11 weeks, it was still significantly better in the CSQ/βARKct mice (Fig. 2 *C* and *D*). The improvement in function was also associated with a significant decrease in the LV weight/body weight in the CSQ/βARKct mice compared with the CSQ mice (Table 1). These data indicate that the βARKct not only improves survival but also markedly impacts and lessens the progression of cardiac failure in this aggressive model of cardiomyopathy.

To determine whether inhibition of βARK1 through overexpression of the βARKct peptide could restore normal βAR signaling, we evaluated receptor–effector coupling in sarcolemmal membranes from 7-week-old transgenic hearts. Total βAR density and the percentage of receptors exhibiting high-affinity β-agonist binding were significantly reduced in the CSQ mice as compared with wild-type mice (Table 2 and Fig. 3*A*). Consistent with lowered βARK1-mediated βAR desensitization, the percentages of βARs in the high-affinity state were restored to normal in the CSQ/βARKct myocardial membranes (Fig. 3*A*). Postreceptor defects have been well characterized in endstage human heart failure. We found a similar defect in CSQ mice, as shown by isoproterenol- and NaF-stimulated adenylyl cyclase activity in the CSQ mice (Table 2). Interestingly, overexpression of the βARKct did not reverse the abnormality in adenylyl cyclase activity compared with the CSQ mice. These data show that the βARKct functions to prevent agonist-induced phosphorylation and desensitization of βARs to maintain normal receptor/G protein coupling; however, overexpression of the βARKct does not alleviate apparent postreceptor defects in the CSQ mouse.

Because βARK1 protein levels and activity are increased in human heart failure, we sought to determine whether myocardial

Table 2. βAR signaling characteristics

Mice	βAR density, fmol/mg protein $n = 4$	Adenylyl cyclase activity, pmol/min per mg protein $n = 5$ Basal	Iso 10^{-4}M	NaF	Fold Iso induction over basal
Wild type	32.8 ± 5.1	24.3 ± 2.5	45.0 ± 5.0*	208.8 ± 34.0‡	1.85
βARKct	32.5 ± 9.4	19.3 ± 1.8	35.6 ± 4.4*	132.0 ± 10.0‡	1.85
CSQ	22.2 ± 2.5	16.6 ± 1.2	22.3 ± 1.6†	75.0 ± 7.0‡	1.34§
CSQ/βARKct	23.5 ± 3.4	15.6 ± 1.2	21.9 ± 1.1†	84.4 ± 6.6‡	1.40§

Iso, isoproterenol. *, $P < 0.01$; †, $P < 0.05$ Iso vs. basal; ‡, $P < 0.0001$ NaF vs. basal; §, $P < 0.05$ CSQ or CSQ/βARKct vs. wild type.

βARK1 levels decrease in the CSQ/βARKct mice as compared with the typically high levels in CSQ mice. Cytosolic myocardial βARK1 protein levels were higher in the CSQ mice than in the wild-type mice (1.3-fold over wild type, $P = 0.05$; $n = 6$), with no significant difference between the CSQ and CSQ/βARKct mice (1.3-fold over wild type vs. 1.5-fold over wild type, respectively; $n = 6$) (Fig. 3*B*). Because of the use of the same promoter in the transgene constructs, CSQ and βARKct expression levels were monitored for potential promoter competition when expressed together. The CSQ and βARKct protein levels assessed by immunoblotting were the same in the single vs. binary transgenic animals (Fig. 3*B*).

Because a marked postreceptor defect persisted in the CSQ/βARKct mice and cardiac function still improved, we tested whether overexpression of the βARKct would affect βAR-stimulated inotropy. We performed cardiac catheterization in intact anesthetized mice. LV contractility (assessed by LV dP/dt_{max}) at baseline in the CSQ/βARKct mice was slightly greater than in the CSQ mice, although it was still significantly less than in wild-type mice (Table 1). As expected, isoproterenol (1 picogram) stimulation gave only a small but significant effect on LV dP/dt_{max} in both the CSQ ($P < 0.05$) and CSQ/βARKct ($P < 0.005$) mice, in contrast to the pronounced wild-type response ($P < 0.0001$). These data are consistent with the biochemical data that show a marked postreceptor abnormality.

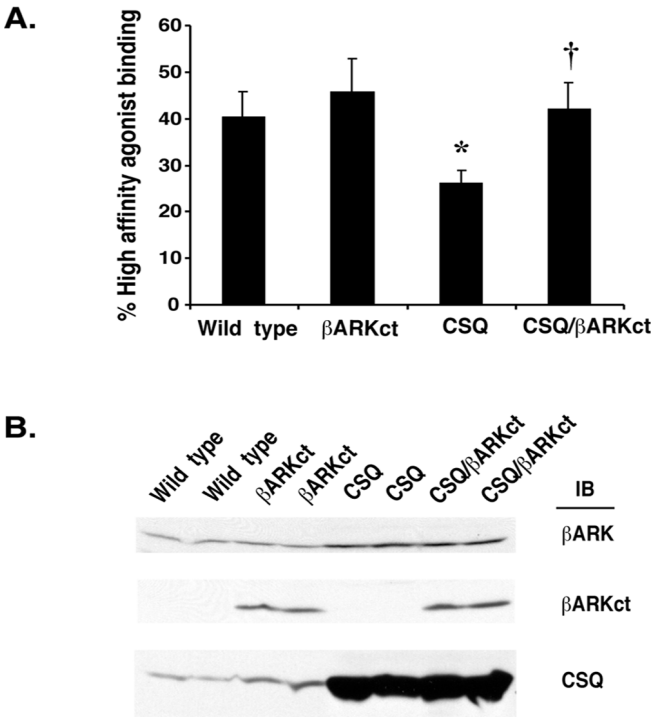

Fig. 3. βARK1 high-affinity β-agonist binding and βARK1 protein expression in CSQ and CSQ/βARKct mice. (*A*) Membrane preparations from left ventricles were used to measure % high-affinity βAR binding. *, $P < 0.05$, CSQ vs. wild type; and †, $P < 0.05$, CSQ/βARKct vs. CSQ. Wild type, $n = 7$; βARKct, $n = 7$; CSQ, $n = 8$; CSQ/βARKct, $n = 6$. (*B*) Immunodetection of βARK1 in cytosolic extracts from wild-type and transgenic hearts at 7 weeks of age. Shown is a representative experiment with two hearts from each gene-targeted mouse. Each heart was immunoblotted (IB) for levels of βARK1 and expression of the transgenes βARKct and CSQ. Protein expression was quantitated by densitometry.

Recent clinical data in human heart failure have shown that the addition of a β-blocker to standard therapy can significantly improve survival in patients with severe heart failure (11–13). To determine whether β-blocker therapy would act in a synergistic fashion with the βARKct, we chronically treated all mice from birth with the selective β_1AR antagonist metoprolol. In a manner consistent with the clinical data, metoprolol also improved survival from 9 ± 1 to 14 ± 1 weeks ($P < 0.0001$) in the CSQ mice (Fig. 4). Remarkably, the combination therapy of βARKct expression and metoprolol treatment in the CSQ mice gave an ≈3-fold increase in the mean survival age. Thus, there was a dramatic lengthening of survival if CSQ mice were treated with both the βARK inhibitor and metoprolol. Metoprolol treatment did provide some functional benefit to the CSQ mice, as seen by a small but significant decrease in chamber size ($P < 0.05$) and increase in cardiac function ($P < 0.05$). Importantly, cardiac function in metoprolol-treated CSQ/βARKct mice was significantly better than in metoprolol-treated CSQ mice, indicating an important positive effect on long-term cardiac function with the βARKct (Table 1). Taken together, these data show that the action of β-blockade is synergistic with the action of the βARKct in failing myocardium.

Discussion

The present study demonstrates that inhibition of βARK1 through cardiac-targeted expression of the βARKct peptide results in a marked improvement in survival in the CSQ model of severe cardiomyopathy. In addition to the significant increase in survival, the βARKct also was able significantly to improve cardiac function, suggesting a mechanism of action that positively affects (i.e., limits) the progression of the myopathic disease. The receptor uncoupling of βAR from G protein usually seen in heart failure was ameliorated through expression of the βARKct, although its expression was not able to reverse the defects observed in adenylyl cyclase activity. Most dramatic, however, was the synergistic action of β-blocker therapy with βARK inhibition, which resulted in a nearly 3-fold increase in survival of the CSQ mice.

Two standard therapies known to increase survival in human heart failure are angiotensin-converting enzyme (ACE) inhibition and β-blocker treatment (14, 15). We demonstrate here that chronic use of the β-blocker metoprolol in addition to βARK

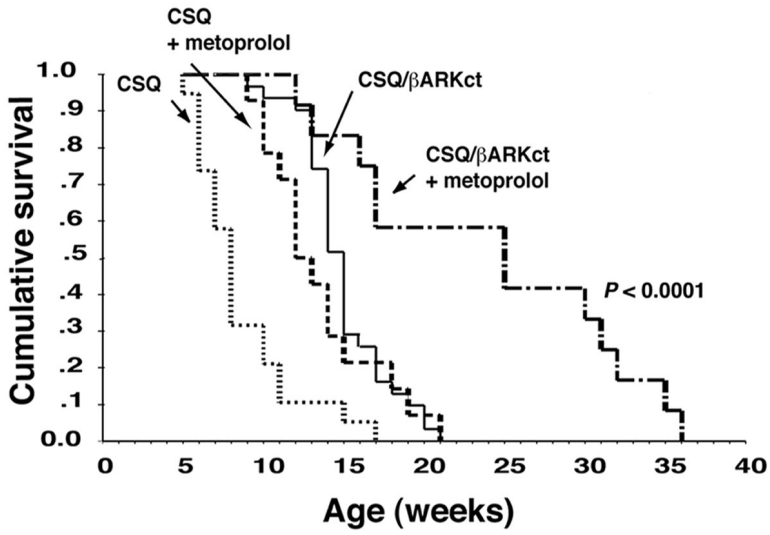

Fig. 4. Survival analysis of the CSQ and CSQ/βARKct mice treated with the β-blocker metoprolol. Kaplan–Meier survival analysis was used to determine the survival probability between the specified genotypes of mice while chronically treated with metoprolol in the drinking water. Mean survival age of the CSQ mice ($n = 14$) receiving no drug was 9 ± 1 weeks vs. 14 ± 2 weeks in the metoprolol-treated CSQ mice ($n = 14$; $P < 0.0005$). Mean survival age of the CSQ/βARKct mice ($n = 31$) receiving no drug was 15 ± 1 weeks vs. 24 ± 3 weeks in the metoprolol-treated CSQ/βARKct mice ($n = 14$; $P < 0.0001$). Curves for wild-type ($n = 20$) and βARKct ($n = 13$) mice treated with metoprolol are not shown but, collectively, they had only one death throughout the course of the study.

inhibition acts synergistically in the CSQ model of cardiomyopathy to increase mean survival age from 9 weeks (with no treatment) to a mean of 24 weeks (with treatment). The increase in survival found when both therapeutic modalities are administered is comparable to the increase in survival observed with β-blocker and ACE therapy in humans (14, 15), and indicates that therapies relating to the βARKct peptide might also provide additional benefits to heart-failure patients who are currently on β-blocker therapy.

In this study, the βARKct peptide delays progression of cardiac dysfunction in the CSQ model of severe cardiomyopathy, as evidenced by the reduced LV dilation and increased cardiac function at 7 weeks in the CSQ/βARKct mice compared with the CSQ mice. Given the aggressive myopathy of the CSQ mice, it is not surprising that the CSQ/βARKct mice eventually progress to myocardial failure and death. This finding is in contrast with the $MLP^{-/-}$/βARKct model of cardiomyopathy, wherein the βARKct prevented completely the progression of the $MLP^{-/-}$ phenotype (4). Reasons for this contrast may be (*i*) the severity of the CSQ phenotype and (*ii*) the inherent differences between the CSQ and $MLP^{-/-}$ models of cardiomyopathy. The lifespan of the $MLP^{-/-}$ model (4) is not appreciably shortened, and survival rate was never studied. In another model of murine cardiomyopathy (cardiac overexpression of G_q), crossbreeding with mice expressing adenylyl cyclase VI could improve heart function; however, survival was never studied (16). Although chronic β-blocker treatment of mice with cardiac overexpression of $G_s\alpha$ diminished the premature death associated with that phenotype (17), our study shows a dramatic increase in survival with the addition of βARK1 inhibition to standard β-blocker therapy.

An interesting finding in our study is that the βARKct was able to decrease some of the hypertrophy in the CSQ/βARKct mice, as seen by the significantly decreased LV weight/body weight at 7 weeks. This finding was especially interesting considering we have recently shown that the βARKct has no affect on preventing the development of pressure-overload hypertrophy (10). Interestingly, ablation of phospholamban in CSQ-expressing mice is capable of rescuing the diminished contractility and myocyte hypertrophy that is typically seen in the CSQ mice (18). These findings may be indicative of the favorable effects on overall cardiac function caused by these manipulations.

One of the most interesting findings of our study was that the βARKct improved survival without affecting the postreceptor defect in the CSQ mice. This defect was evident by the persistent abnormality in isoproterenol-stimulated adenylyl cyclase and LV dP/dt_{max} in the CSQ/βARKct mice. Considerable controversy continues concerning whether inotropic therapies are detrimental when used in heart failure cases, especially considering the dismal results of trials in patients with inotropic agents (19). Our data clearly show that, whereas the βARKct acts to normalize βAR G protein coupling as shown by the improvement in high-affinity agonist binding, it only acts mildly to enhance the inotropic state of the heart in this model. This result is not surprising, given the significant postreceptor defect and the inability of the CSQ mice to effectively release Ca^{2+} (necessary for an increase in contractility) from the sarcoplasmic reticulum (7). However, the biochemical data demonstrate that the βARKct functions to inhibit βARK1-mediated receptor effects, as shown by the increased βAR density and the percentage of high-affinity agonist binding sites. Nevertheless, βARKct expression through noninotropic means significantly improved cardiac function, delayed overt failure, and improved survival of the CSQ mice.

One possible mechanism for the beneficial effect of the βARKct is decreased desensitization of other G protein-coupled receptors, such as endothelin and angiotensin receptors. Although previous studies have shown the benefits of blocking both angiotensin (20) and endothelin (21) receptors, inhibiting βARK1, as we have done here, would act to enhance angiotensin and endothelin signaling; therefore, inhibiting βARK1 on these receptors does not seem to be the likely mechanism of action. Because the βARKct peptide inhibits βARK1 through the sequestration of Gβγ-subunits, it is possible that the mechanism of action of the βARKct for delaying the progression of the CSQ phenotype is caused, in part, by the inhibition of other Gβγ-dependent pathways such as adenylyl cyclase (22), phospholipase C-β (PLCβ) (23), mitogen-activated protein (MAP) kinase (23), or phosphatidylinositol (PI) 3-kinase (23). MAP kinase and PI 3-kinase activities have recently been shown to be influenced by

activated $G\beta\gamma$-βARK1 (24, 25), which, in this model of $G\beta\gamma$ sequestration, would be inhibited by the overexpression of the βARKct.

Whereas chronic metoprolol treatment markedly improved survival in CSQ/βARKct mice, it had no additional benefits to cardiac function. These data suggest that the mechanism of action of the βARKct is distinctly different from that of metoprolol. β-blocker therapy is known to be antiarrhythmic, and the CSQ model has very prominent arrhythmias with marked abnormalities in ion-channel function (7, 26). It is likely that the synergistic action of the two therapeutic approaches is related to the positive influence on cardiac function of the βARKct and the reduction in arrhythmias with metoprolol. Additionally, the combination of βARK inhibition and β-blockade may differentially affect β_1 and β_2AR subtype signaling, given that it has recently been shown in transgenic mouse models that enhanced β_1AR signaling is deleterious (27), whereas enhanced β_2AR signaling can be beneficial (28).

In summary, this study demonstrates that the inhibition of βARK1 through the expression of the βARKct peptide is able to increase survival and delay the progression of myocardial failure in the CSQ model of cardiomyopathy. That the βARKct is able to significantly improve multiple and different models of cardiomyopathy and that its benefits are synergistic with standard β-blocker therapy suggest that drugs which inhibit βARK1 might serve as an important new class of therapeutic agents for the treatment of human heart failure.

We gratefully acknowledge Dr. Lan Mao for her expertise in microsurgery and echocardiography and the technical support of Kyle Shotwell for the β-adrenergic binding experiments. This work was supported in part by National Institutes of Health Grants HL61558 (to H.A.R.), HL61690 (to W.J.K.), HL28556 (to L.R.J.), and HL16037 (to R.J.L.). R.J.L. is an investigator of the Howard Hughes Medical Institute. H.A.R. is a recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

1. Lefkowitz, R. J. (1993) *Cell* **74,** 409–412.
2. Bristow, M. R., Ginsburg, R., Minobe, W., Cubicciotti, R. S., Sageman, W. S., Lurie, K., Billingham, M. E., Harrison, D. C. & Stinson, E. B. (1982) *N. Engl. J. Med.* **307,** 205–211.
3. Ungerer, M., Bohm, M., Elce, J. S., Erdmann, E. & Lohse, M. J. (1993) *Circulation* **87,** 454–463.
4. Rockman, H. A., Chien, K. R., Choi, D. J., Iaccarino, G., Hunter, J. J., Ross, J., Jr., Lefkowitz, R. J. & Koch, W. J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 7000–7005.
5. Koch, W. J., Rockman, H. A., Samama, P., Hamilton, R. A., Bond, R. A., Milano, C. A. & Lefkowitz, R. J. (1995) *Science* **268,** 1350–1353.
6. White, D. C., Hata, J. A., Shah, A. S., Glower, D. D., Lefkowitz, R. J. & Koch, W. J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 5428–5433. (First Published April 25, 2000; 10.1073/pnas.090091197)
7. Jones, L. R., Suzuki, Y. J., Wang, W., Kobayashi, Y. M., Ramesh, V., Franzini-Armstrong, C., Cleemann, L. & Morad, M. (1998) *J. Clin. Invest.* **101,** 1385–1393.
8. Cho, M. C., Rapacciuolo, A., Koch, W. J., Kobayashi, Y., Jones, L. R. & Rockman, H. A. (1999) *J. Biol. Chem.* **274,** 22251–22256.
9. Esposito, G., Santana, L. F., Dilly, K., Cruz, J. D., Mao, L., Lederer, W. J. & Rockman, H. A. (2000) *Am. J. Physiol.* **279,** H3101–H3112.
10. Choi, D. J., Koch, W. J., Hunter, J. J. & Rockman, H. A. (1997) *J. Biol. Chem.* **272,** 17223–17229.
11. Packer, M., Bristow, M. R., Cohn, J. N., Colucci, W. S., Fowler, M. B., Gilbert, E. M. & Shusterman, N. H. (1996) *N. Engl. J. Med.* **334,** 1349–1355.
12. Hjalmarson, A., Goldstein, S., Fagerberg, B., Wedel, H., Waagstein, F., Kjekshus, J., Wikstrand, J., El Allaf, D., Vitovec, J., Aldershvile, J., *et al*. (2000) *J. Am. Med. Assoc.* **283,** 1295–1302.
13. Hart, S. M. (2000) *Ann. Pharmacother.* **34,** 1440–1451.
14. Packer, M. (1997) *Am. J. Cardiol.* **80,** 46L–54L.
15. Garg, R. & Yusuf, S. (1995) *J. Am. Med. Assoc.* **273,** 1450–1456.
16. Roth, D. M., Gao, M. H., Lai, N. C., Drumm, J., Dalton, N., Zhou, J. Y., Zhu, J., Entrikin, D. & Hammond, H. K. (1999) *Circulation* **99,** 3099–3102.
17. Asai, K., Yang, G. P., Geng, Y. J., Takagi, G., Bishop, S., Ishikawa, Y., Shannon, R. P., Wagner, T. E., Vatner, D. E., Homcy, C. J. & Vatner, S. F. (1999) *J. Clin. Invest.* **104,** 551–558.
18. Sato, Y., Kiriazis, H., Yatani, A., Schmidt, A. G., Hahn, H., Ferguson, D. G., Sako, H., Mitarai, S., Honda, R., Mesnard-Rouiller, L., *et al*. (2001) *J. Biol. Chem.* **276,** 9392–9399.
19. Betkowski, A. S. & Hauptman, P. J. (2000) *Curr. Opin. Cardiol.* **15,** 293–303.
20. Pitt, B., Segal, R., Martinez, F. A., Meurers, G., Cowley, A. J., Thomas, I., Deedwania, P. C., Ney, D. E., Snavely, D. B. & Chang, P. I. (1997) *Lancet* **349,** 747–752.
21. Sakai, S., Miyauchi, T., Kobayashi, M., Yamaguchi, I., Goto, K. & Sugishita, Y. (1996) *Nature (London)* **384,** 353–355.
22. Tang, W. J. & Gilman, A. G. (1992) *Cell* **70,** 869–872.
23. Clapham, D. E. & Neer, E. J. (1993) *Nature (London)* **365,** 403–406.
24. Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudsley, S., Della Rocca, G. J., Lin, F., Kawakatsu, H., Owada, K., Luttrell, D. K., *et al*. (1999) *Science* **283,** 655–661.
25. Naga Prasad, S. V., Esposito, G., Mao, L., Koch, W. J. & Rockman, H. A. (2000) *J. Biol. Chem.* **275,** 4693–4698.
26. Knollmann, B. C., Knollmann-Ritschel, B. E., Weissman, N. J., Jones, L. R. & Morad, M. (2000) *J. Physiol. (London)* **525** Pt. 2**,** 483–498.
27. Engelhardt, S., Hein, L., Wiesmann, F. & Lohse, M. J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 7059–7064.
28. Liggett, S. B., Tepe, N. M., Lorenz, J. N., Canning, A. M., Jantz, T. D., Mitarai, S., Yatani, A. & Dorn, G. W. (2000) *Circulation* **101,** 1707–1714.