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N. P. L. Rolim, A. Medeiros, K. T. Rosa, K. C. Mattos, M. C. Irigoyen, E. M. Krieger, J. E. Krieger, C. E. Negrao and P. C. Brum

*Physiol Genomics*, May 11, 2007; 29 (3): 246-252.

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**Differential cardioprotective/cardiotoxic effects mediated by {beta}-adrenergic receptor subtypes**

D. Bernstein, G. Fajardo, M. Zhao, T. Urashima, J. Powers, G. Berry and B. K. Kobilka

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## Abnormal cardiac function associated with sympathetic nervous system hyperactivity in mice

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DANIEL BERNSTEIN,<sup>4</sup> AND BRIAN KOBILKA<sup>2,5</sup>

<sup>1</sup>Department of Pathology, Veterans Administration Medical Center, Palo Alto 94305; and <sup>2</sup>Departments of Medicine and Molecular and Cellular Physiology, <sup>3</sup>Department of Anesthesia, <sup>4</sup>Division of Cardiology, Department of Pediatrics, and <sup>5</sup>Howard Hughes Medical Institute, Stanford University, Stanford, California 94305

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**Brum, Patricia C., Jon Kosek, Andrew Patterson, Daniel Bernstein, and Brian Kobilka.** Abnormal cardiac function associated with sympathetic nervous system hyperactivity in mice. *Am J Physiol Heart Circ Physiol* 283: H1838–H1845, 2002. First published July 26, 2002; 10.1152/ajpheart.01063.2001.— $\alpha_{2A}$ -Adrenergic receptors (ARs) in the midbrain regulate sympathetic nervous system activity, and both  $\alpha_{2A}$ -ARs and  $\alpha_{2C}$ -ARs regulate catecholamine release from sympathetic nerve terminals in cardiac tissue. Disruption of both  $\alpha_{2A}$ - and  $\alpha_{2C}$ -ARs in mice leads to chronically elevated sympathetic tone and decreased cardiac function by 4 mo of age. These knockout mice have increased mortality, reduced exercise capacity, decreased peak oxygen uptake, and decreased cardiac contractility relative to wild-type controls. Moreover, we observed significant abnormalities in the ultrastructure of cardiac myocytes from  $\alpha_{2A}/\alpha_{2C}$ -AR knockout mice by electron microscopy. Our results demonstrate that chronic elevation of sympathetic tone can lead to abnormal cardiac function in the absence of prior myocardial injury or genetically induced alterations in myocardial structural or functional proteins. These mice provide a physiologically relevant animal model for investigating the role of the sympathetic nervous system in the development and progression of heart failure.

$\alpha_2$ -adrenergic receptor; knockout mice; heart failure

HEART FAILURE is a common end point for many forms of cardiovascular disease and a significant cause of morbidity and mortality. The development of end-stage heart failure often involves an initial insult to the myocardium that reduces cardiac output and leads to a compensatory increase in sympathetic nervous system activity. There is a growing body of evidence that, while beneficial acutely, chronic exposure of the heart to elevated levels of catecholamines released from sympathetic nerve terminals and the adrenal gland may lead to further pathological changes in the heart, resulting in a continued elevation of sympathetic tone

and a progressive deterioration in cardiac function (4, 5, 9, 34). However, direct experimental evidence that the sympathetic nervous system plays a predominant role in the development of heart failure is lacking, and there are no suitable murine models of heart failure based on elevated sympathetic nervous system activity without concomitant alterations of myocardial structural or functional proteins. Most murine models of heart failure are based on the disruption of genes for cardiac specific proteins (2) or the use of cardiac-specific promoters to overexpress proteins that disrupt myocyte function (10, 12, 13, 15, 21, 33, 42). Whereas these models have been used to test novel approaches for the treatment of heart failure (17, 20, 25, 37, 38, 41, 43), they may not accurately reflect the pathogenesis of this disorder in humans. Here we report a model of heart failure based on the disruption of genes that regulate sympathetic nervous system activity.

There are three  $\alpha_2$ -adrenergic receptor ( $\alpha_2$ -AR) subtypes:  $\alpha_{2A}$ ,  $\alpha_{2B}$ , and  $\alpha_{2C}$ .  $\alpha_2$ -ARs regulate the sympathetic nervous system in several ways.  $\alpha_{2A}$ -ARs in the brain stem regulate sympathetic tone (1, 29), and both  $\alpha_{2A}$ -AR and  $\alpha_{2C}$ -AR act as presynaptic autoreceptors regulating catecholamine release in the murine atria (18). We have previously reported that disruption of both  $\alpha_{2A}$ - and  $\alpha_{2C}$ -ARs in mice leads to chronically elevated sympathetic tone (18). Here we report that  $\alpha_{2A}/\alpha_{2C}$ -AR knockout (KO) mice have abnormal cardiac function by 4 mo of age. These mice have reduced exercise capacity, decreased peak oxygen uptake, and decreased cardiac contractility relative to wild-type controls. Moreover, we observed evidence of direct myocyte damage by electron microscopy. Our results provide direct evidence that elevated sympathetic nervous system activity can lead directly to pathological changes in the heart. Moreover, they provide evidence that subtype selective  $\alpha_2$ -AR agonists may be clinically beneficial in the prevention and treatment of heart failure.

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## MATERIALS AND METHODS

**Generation of  $\alpha_2$ -ARKO mice.** Heterozygous  $\alpha_{2A}$ -ARKO mice (1) and  $\alpha_{2C}$ -ARKO mice (28) were bred to wild-type C57Bl6/J mice for five successive generations to produce strains of mice having a uniform, predominantly C57Bl6/J genetic background.  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice were generated by mating the C57Bl6/J  $\alpha_{2A}$ -AR homozygous KO mice to homozygous C57Bl6/J  $\alpha_{2C}$ -ARKO mice. The resulting F1 generation of compound heterozygous mice were subsequently intercrossed to generate F2 mice with all possible combinations of  $\alpha_{2A}$ - and  $\alpha_{2C}$ -AR gene disruptions. The  $\alpha_{2A}$ -ARKO,  $\alpha_{2A}/\alpha_{2C}$ -ARKO, and wild-type mice produced from this cross were bred to establish the lines of mice used in these experiments. Genotypes were determined by PCR on genomic DNA obtained from tail biopsies using primers to detect the intact and disrupted genes.

**Graded treadmill exercise test.** Exercise capacity, estimated by the total distance run, and peak oxygen uptake ( $\dot{V}O_2$ ) values were recorded using a graded treadmill exercise protocol for mice, as previously described (11). Briefly, a four-lane Columbus Instruments Simplex II mouse treadmill fitted with a metabolic analysis system consisting of OxyMax oxygen and carbon dioxide gas analyzers (Columbus Instruments; Columbus, OH) was used. Mice were placed in the exercise chamber and allowed to acclimatize for at least 30 min. The treadmill activity was initiated at 7.5 m/min and 4° inclination and was increased to 10 m/min and 6° inclination 3 min later. Treadmill speed and inclination were then increased by 2.5 m/min and 2° inclination (considered as 1 workload unit) every 3 min thereafter until exhaustion. The graded treadmill exercise test was performed serially in wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice at 1, 2, 3, and 4 mo of age. Additional graded treadmill exercise tests were performed on a separate group of 6-mo-old wild-type,  $\alpha_{2A}$ -ARKO, and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.

**Cardiovascular measurements.** Blood pressure and heart rate were determined noninvasively using a computerized tail-cuff system (BP 2000 Visitech Systems) described elsewhere (19). Mice were acclimatized to the apparatus during daily sessions over 6 days, 1 wk before the final measurements were obtained. Blood pressure measurements were obtained on the last week of each month. Blood pressure tail-cuff measurement in the fourth month was confirmed by direct measurement via arterial catheterization at 4 and 6 mo.

Blood pressure and heart rate measurements were also obtained from a chronic indwelling carotid arterial catheter (11). After 24 h of recovery, blood pressure and heart rate were recorded with a Gould eight-channel recorder and digitized on a Crystal Biotech Dataflow system (Hopkinton, MA). Heart rate measurements were determined on-line, derived from the pressure recordings. Baseline hemodynamics were continuously recorded in conscious freely moving mice for 1 h after the animal was placed in the study cage. By 30 min, the mice were resting quietly on their bedding, and blood pressure readings were stable. Blood pressure readings were averaged over the last 30 min of recording. To examine the heart rate response to  $\beta$ -AR stimulation, *l*-isoproterenol hydrochloride (3  $\mu$ g/kg ia) was administered.

To perform left ventricular catheterization, mice were anesthetized with isoflurane (1.25–1.75%) and placed on a warmed table. A 1.8-Fr high-fidelity catheter-tipped micromanometer (Millar Instruments; Houston, TX) was inserted into the aorta via the right carotid artery and advanced into the left ventricle under continuous monitoring of the pressure waveform. Pressure signals were digitized at a sampling

rate of 1,000 Hz and recorded with a Data Q system. The left ventricular pressure was recorded pre- and postinjection of propranolol (30 mg/kg ip). Noninvasive cardiac function was assessed by two-dimensional guided M-mode echocardiography of isoflurane-anesthetized 1- and 6-mo-old wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.

**Structural analysis.** Myocardial cytoarchitecture was examined using standardized electron microscopic methods. Hearts were fixed by immersion in glutaraldehyde. Sections from the left ventricular free wall from two wild-type and three  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice were examined. The pathologist was blinded to the genotype throughout the sample preparation and analysis. Ten electron micrographs were taken from each heart and graded for evidence of abnormal myocyte structure: loss of myofibril integrity, mitochondrial swelling and loss of mitochondrial integrity, and vacuolization. Micrographs were scored on a scale of 0–4 with 0 being “normal” and 4 being the most abnormal.

To assess myocyte width, hearts were embedded in paraffin and sectioned for routine histological processing. Sections were stained with hematoxylin and eosin for examination with a light microscope. Myocyte width was measured from myocytes in the left ventricular free wall with a computer-assisted morphometric system (Leica Quantimet 500). For each myocyte containing a nucleus visible in the field, a single transverse measurement of width, passing through the nucleus, was obtained. Data were obtained from 7 wild-type and 10  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice. Ten cells were measured from each heart.

**Statistical analysis.** All values are expressed as means  $\pm$  SE. For single measurement variables (direct blood pressure at 4 mo of age, resting heart rate, heart rate after isoproterenol administration, sympathetic tone and injure score), comparisons between wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice were performed using Student's *t*-test. For multiple measurement variables ( $\dot{V}O_2$ , distance run, cardiac contractility and relaxation, echocardiographic characteristics, direct blood pressure at 6 mo of age and tail-cuff measurements), comparisons were performed using two-way ANOVA with post hoc testing by Fisher's protected least-significant-difference test.

## RESULTS

**Decreased exercise capacity in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.** To investigate the role of the sympathetic nervous system in heart failure, we generated two lines of  $\alpha_2$ -ARKO mice on a C57Bl6 background: mice lacking the  $\alpha_{2A}$ -AR gene ( $\alpha_{2A}$ -ARKO mice) and mice lacking both the  $\alpha_{2A}$ - and  $\alpha_{2C}$ -AR genes ( $\alpha_{2A}/\alpha_{2C}$ -ARKO mice).  $\alpha_{2A}$ -ARKO mice have elevated sympathetic tone due to loss of  $\alpha_{2A}$ -AR regulation in the midbrain as well as loss of presynaptic  $\alpha_{2A}$ -AR autoinhibition (1). However, sympathetic tone is further elevated in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice because of the complete loss of presynaptic autoinhibition. We therefore carried out a detailed study of cardiovascular performance in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice from 1 to 6 mo of age and made selective comparisons with the  $\alpha_{2A}$ -ARKO mice.

Exercise capacity correlates well with cardiac function, and exercise intolerance is a common method of characterizing cardiac reserve and function in humans with chronic heart failure (3, 39). Indeed, peak  $\dot{V}O_2$  at exhaustive exercise is one of the most important tests to determine whether patients are sufficiently debili-

tated by heart failure to seriously consider cardiac transplant (30, 32).

A cohort of wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice was subjected to a graded treadmill exercise protocol (36) at 1, 2, 3, and 4 mo of age. Mice were exercised until physical exhaustion, and peak  $\dot{V}O_2$  and total distance run were determined. There were no differences in peak  $\dot{V}O_2$  and distance run between 1-mo-old wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice (Fig. 1, A and C, respectively). However, 3- and 4-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice showed a significant decrease in peak  $\dot{V}O_2$  and distance run compared with wild-type mice (Fig. 1, A and C, respectively).

On the basis of these results, we performed additional exercise studies on different groups of wild-type,  $\alpha_{2A}$ -ARKO, and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice at 6 mo of age (Fig. 1, B and D). This second set of studies was done to verify that disruption of genes for both the  $\alpha_{2A}$ - and  $\alpha_{2C}$ -subtypes is responsible for the development of cardiac dysfunction. We chose 6 mo for this analysis because we observed significant mortality in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice at 6 mo of age (35% for  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice compared with 5% for wild-type mice,  $P < 0.013$ ). The run distances for  $\alpha_{2A}/\alpha_{2C}$ -ARKO and  $\alpha_{2A}$ -ARKO mice

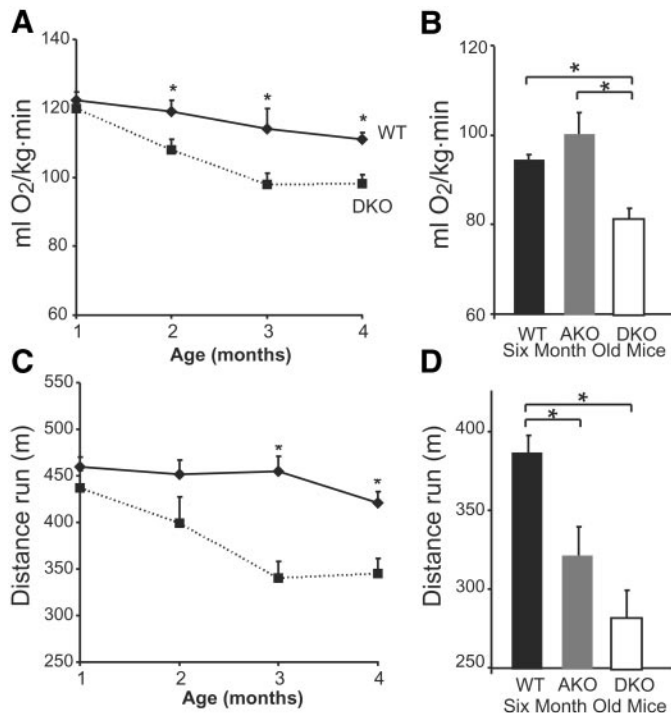


Fig. 1. Exercise capacity of wild-type (WT),  $\alpha_{2A}$ -adrenoceptor (AR) knockout (AKO), and  $\alpha_{2A}/\alpha_{2C}$ -AR knockout (DKO) mice. A: peak oxygen uptake ( $\dot{V}O_2$ ) as a function of age. B: maximal distance run as a function of age. Data were obtained for a cohort of WT and DKO mice at 1–4 mo of age. Data were obtained for a separate cohort of WT, AKO, and DKO mice at 6 mo of age. Data are presented as means  $\pm$  SE. The number of mice studied ( $n$ ) in the 1- to 4-mo period was 13 WT and 14 DKO. The statistical analysis performed was two-way ANOVA with post hoc testing by Fisher's protect least-significant-difference (PLSD) test. At 6 mo,  $n = 5$  WT, 5 AKO, and 5 DKO mice. The statistical analysis performed was one-way ANOVA with post hoc testing by Fisher's PLSD test. \*Significant difference between groups ( $P \leq 0.05$ ).

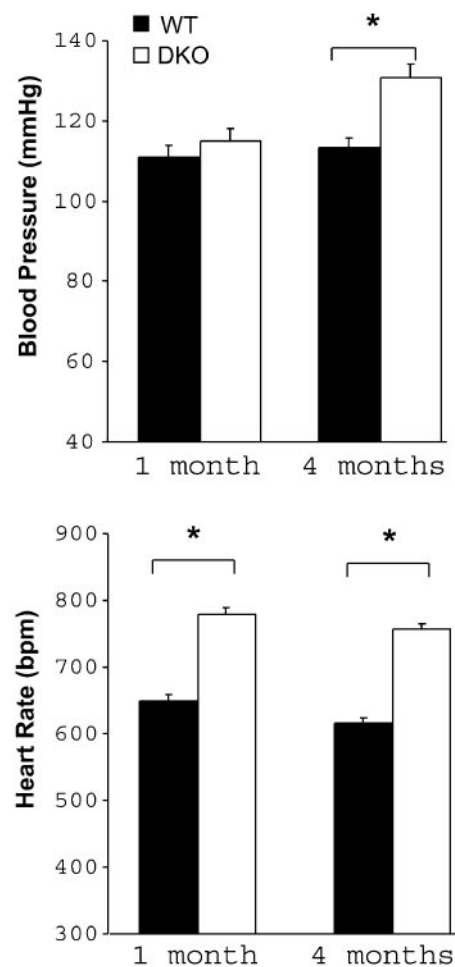


Fig. 2. Noninvasive heart rate and systolic blood pressure measurements. Tail-cuff measurements in WT and DKO mice at 1 and 4 mo of age are shown. A: blood pressure (in mmHg); B: heart rate [in beats/min (bpm)]. Data are presented as means  $\pm$  SE;  $n = 10$  WT and 6 DKO. The statistical analysis performed was two-way ANOVA with post hoc testing by Fisher's PLSD test. \*Significant difference between WT and DKO mice ( $P \leq 0.05$ ).

were significantly shorter than the run distance for wild-type mice (Fig. 1D). However, only  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice showed a significant reduction in peak  $\dot{V}O_2$  relative to wild-type mice (Fig. 1B). Thus the degree of functional impairment in  $\alpha_{2A}$ -ARKO mice is less compared with  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.

*Heart rate and blood pressure of  $\alpha_{2A}$ -ARKO and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.* The effect of  $\alpha_{2A}/\alpha_{2C}$ -AR gene disruption on basal cardiovascular hemodynamics during the period of study was determined by tail-cuff measurements at 1 and 4 mo of age. Baseline systolic blood pressure was not different among the groups at 1 mo, but it was significantly higher in 4-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice than in wild-type mice (Fig. 2A). In addition,  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice showed a significantly higher baseline heart rate at both 1 and 4 mo when compared with wild-type mice (Fig. 2B).

We obtained resting hemodynamic data using an intra-arterial catheter on 4- and 6-mo-old wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice and on 6-mo-old  $\alpha_{2A}$ -ARKO

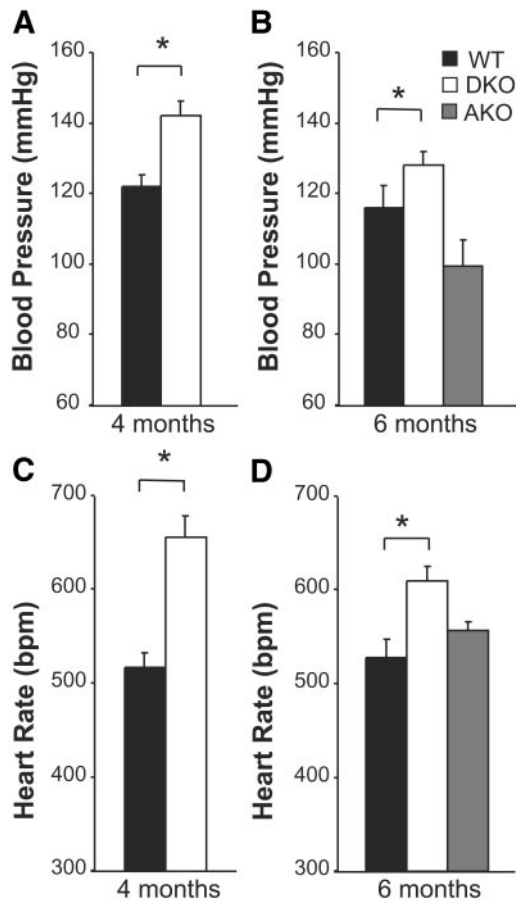


Fig. 3. Heart rate and blood pressure determined from an indwelling arterial catheter. Catheterization was performed as described in MATERIALS AND METHODS. A and C: baseline systolic blood pressure (A) and heart rate (C) in WT and DKO mice at 4 mo of age; B and D: blood pressure (B) and heart rate (D) in WT, AKO, and DKO mice at 6 mo of age. Data are presented as means  $\pm$  SE;  $n = 4$  WT, 4 AKO, and 4 DKO. \*Significant difference between groups ( $P \leq 0.05$ ).

mice (Fig. 3). Consistent with tail-cuff blood pressure measurements at 4 mo, 4- and 6-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice have elevated baseline systolic blood pressure compared with wild-type mice (Fig. 3), and the heart rate was significantly elevated in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice at 4 mo of age. No significant elevation of heart rate or blood pressure was observed in  $\alpha_{2A}$ -ARKO mice (Fig. 3). Whereas hypertension has been shown to lead to cardiac hypertrophy and ultimately heart failure (8, 14), the degree of hypertension observed in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice is relatively mild, and we observed no evidence of cardiac hypertrophy as determined by heart weight/body weight (data not shown). Thus hypertension is not likely to be the etiology of heart failure in these mice.

$\beta$ -AR downregulation has been proposed as a mechanism by which chronic sympathetic stimulation may lead to decreased cardiac performance (7, 16, 23, 26, 35, 40). To verify that  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice have elevated sympathetic tone, we measured the heart rate after pharmacological blockade of muscarinic receptors with atropine in the presence and absence of the  $\beta$ -AR

antagonist propranolol. This difference in heart rate (heart rate after atropine – heart rate after atropine and propranolol) reflects the basal sympathetic tone. We observed a significant increase in cardiac sympathetic tone in 4-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice compared with wild-type controls ( $181 \pm 8$  vs.  $137 \pm 13$  beats/min, respectively). This is consistent with the high levels of circulating catecholamines in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice previously observed in our laboratory (18). However, there was no difference in the maximal chronotropic response to isoproterenol between wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice ( $735 \pm 17$  vs.  $759 \pm 14$  beats/min, respectively). Thus there is no functional evidence for  $\beta$ -AR desensitization or downregulation as a result of chronic elevated sympathetic tone.

*Abnormal contractile function in the hearts of  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.* The exercise studies provided indirect evidence for decreased cardiac performance in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice. These results were confirmed by examining contractile function in the three strains of mice at 4 mo of age by directly monitoring the change in left ventricular pressure over time ( $dP/dt$ ) using a Millar catheter. Figure 4 shows a comparison of the maximal (A) and minimal (B)  $dP/dt$  values for the each strain before and after the administration of the  $\beta$ -AR antagonist propranolol to block the effects of endogenous catecholamines. Although the  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice showed a trend toward decreased baseline cardiac contractility compared with wild-type littermates, this difference was not significant. However, after treatment

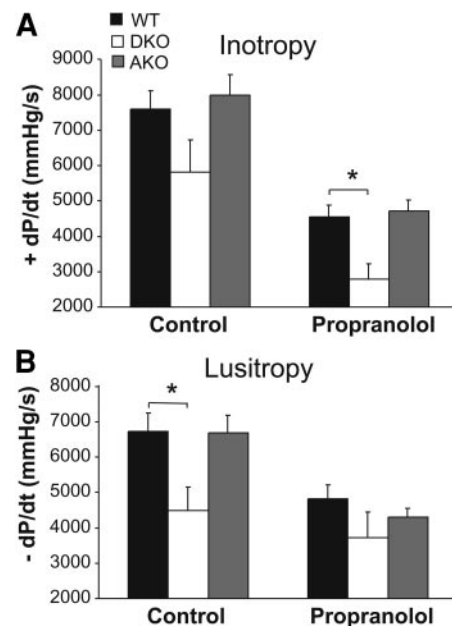


Fig. 4. Inotropic and lusitropic function of hearts from WT, AKO, and DKO mice at 4 mo of age. Left ventricular maximal contractility ( $+dP/dt$ ; A) and relaxation ( $-dP/dt$ ; B) pre- and post- $\beta$ -adrenergic blockade by propranolol (30 mg/kg ip) are shown. Left ventricular pressures in anesthetized mice were measured with a Millar 1.8-Fr catheter. Data are presented as means  $\pm$  SE;  $n = 5$  WT, 5 AKO, and 5 DKO. The statistical analysis performed was two-way ANOVA with post hoc testing by Fisher's PLSD test. \*Significant difference between groups ( $P \leq 0.05$ ).

with propranolol, contractile function in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice was significantly reduced relative to wild-type and  $\alpha_{2A}$ -ARKO mice (Fig. 4A). The result cannot be explained by differences in heart rate, because there were no significant differences between the heart rates of wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice treated with propranolol ( $592 \pm 16$  vs.  $614 \pm 20$  beats/min, respectively).  $\alpha_{2A}$ -ARKO mice treated with propranolol had a slightly lower heart rate ( $538 \pm 8$  beats/min) than wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice; however, this would not be expected to artifactually increase the maximal dP/dt.

Lusitropic function was also abnormal in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice. The rate of relaxation as reflected in the minimal dP/dt was significantly lower in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice relative to wild-type and  $\alpha_{2A}$ -ARKO mice (Fig. 4B). A similar trend was observed in mice after treatment with propranolol; however, this difference was not significant.

Consistent with dP/dt measurements, echocardiography on age-matched wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice at 1 and 6 mo of age provided evidence of abnormal cardiac function in 6-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice. The left ventricular fractional shortening was significantly impaired, whereas the systolic and diastolic dimensions were significantly increased in 6-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice when compared with age-matched wild-type and 1-mo-old wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice (Table 1).

**Abnormal myocyte structure in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice.** There was no obvious difference in the hearts of wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice at 4 and 6 mo as determined by heart weight-to-body weight ratios. However, electron microscopy revealed marked abnormalities of myocyte ultrastructure. Figure 5 shows the heart ultrastructure in both  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice (A and B) and wild-type mice (C and D) at 4 mo of age. We observed myofibrillar disarray, mitochondrial degeneration, and vacuolization in the heart of  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice compared with wild-type mice. Electron microscope sections of hearts from wild-type and  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice were processed and analyzed by a pathologist blinded to the genotype. Myocytes from  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice had a significantly higher injury score than those from

wild-type mice (Fig. 5E). Surprisingly, no significant evidence of fibrosis was detected by trichrome staining of hearts from 4-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice when compared with hearts from wild-type mice. However, there was evidence for myocyte hypertrophy in ventricles from 4-mo-old  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice. The averaged myocyte width for wild-type mice was  $21.03 \pm 0.56 \mu\text{m}$  compared with  $27.36 \pm 0.41 \mu\text{m}$  for  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice ( $P < 0.01$ ).

## DISCUSSION

Previous studies have suggested a role for chronic sympathetic activation in the pathogenesis of heart failure. In clinical practice, beta-blockers have become an established form of therapy for chronic heart failure (6). Our studies show that by 4 mo of age,  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice have evidence of elevated sympathetic tone including a higher baseline heart rate and a modest elevation in systolic blood pressure. As a result, these mice also show signs of cardiac dysfunction: decreased maximal exercise capacity and contractility. We did not observe a significant difference in heart weight when comparing  $\alpha_{2A}/\alpha_{2C}$ -ARKO and wild-type mice. However, electron microscopy revealed evidence of significant cardiac myocyte injury in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice, and light microscopy indicates that the remaining myocytes are enlarged. At 6 mo of age, we observed a significant reduction in fractional shortening by echocardiography in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice. These findings are most consistent with direct myocardial damage due to chronically elevated sympathetic tone. However, the sympathetic nervous system also modulates the activity of the renin-angiotensin system (24), which may contribute to the pathogenesis of heart failure of these mice. Moreover, we cannot exclude a contribution of elevated blood pressure or an as-yet-undetermined effect of the disruption of both the  $\alpha_{2A}$ - and  $\alpha_{2C}$ -ARs on cardiac function.

Functional differences observed in  $\alpha_{2A}$ -ARKO mice were much less severe and consisted of a modest decrease in the distance run without a change in peak  $\text{VO}_2$  or cardiac contractility. This is somewhat surprising because the  $\alpha_{2A}$ -AR plays the predominant role in regulating sympathetic nervous system function.  $\alpha_{2A}$ -ARs in the midbrain regulate sympathetic tone (1, 29), and presynaptic catecholamine release in the heart is primarily regulated by this subtype (18). In contrast,  $\alpha_{2C}$ -ARKO mice have normal baseline heart rate and blood pressure, have a normal hypotensive response to an  $\alpha_2$ -AR agonist (27), and do not develop heart failure. Moreover, presynaptic  $\alpha_{2C}$ -ARs are less effective than  $\alpha_{2A}$ -ARs in inhibiting catecholamine release (18). Nevertheless, the data in the present study suggest that residual presynaptic autoinhibition mediated by the  $\alpha_{2C}$ -AR is sufficient to prevent or delay myocardial injury in  $\alpha_{2A}$ -ARKO mice. The presynaptic  $\alpha_{2C}$ -AR may be more important in regulating catecholamine release at low frequency sympathetic nerve activity (18), such as during periods of rest. Nonselective

Table 1. Echocardiographic characteristics of 1- and 6-mo-old WT and DKO mice

	WT		DKO	
	1 mo	6 mo	1 mo	6 mo
FS, %	$56 \pm 3$	$52 \pm 4$	$63 \pm 3$	$29 \pm 3^{*\dagger}$
ESD, mm	$1.7 \pm 0.1$	$2.1 \pm 0.3$	$1.5 \pm 0.2$	$3.6 \pm 0.3^{*\dagger}$
EDD, mm	$3.9 \pm 0.3$	$4.2 \pm 0.3$	$4.0 \pm 0.3$	$5.1 \pm 0.3^{*\dagger}$
n	5	4	4	13

Data presented are means  $\pm$  SE; n = no. of mice. FS, left ventricular (LV) fractional shortening; ESD, LV end-systolic dimension; EDD, LV end-diastolic dimension. The statistical analysis performed was two-way ANOVA with post hoc testing by Fisher's protected least-significant-difference test. \*Significant difference from WT mice ( $P \leq 0.05$ );  $\dagger$ significant difference from 1-mo-old mice ( $P < 0.05$ ).

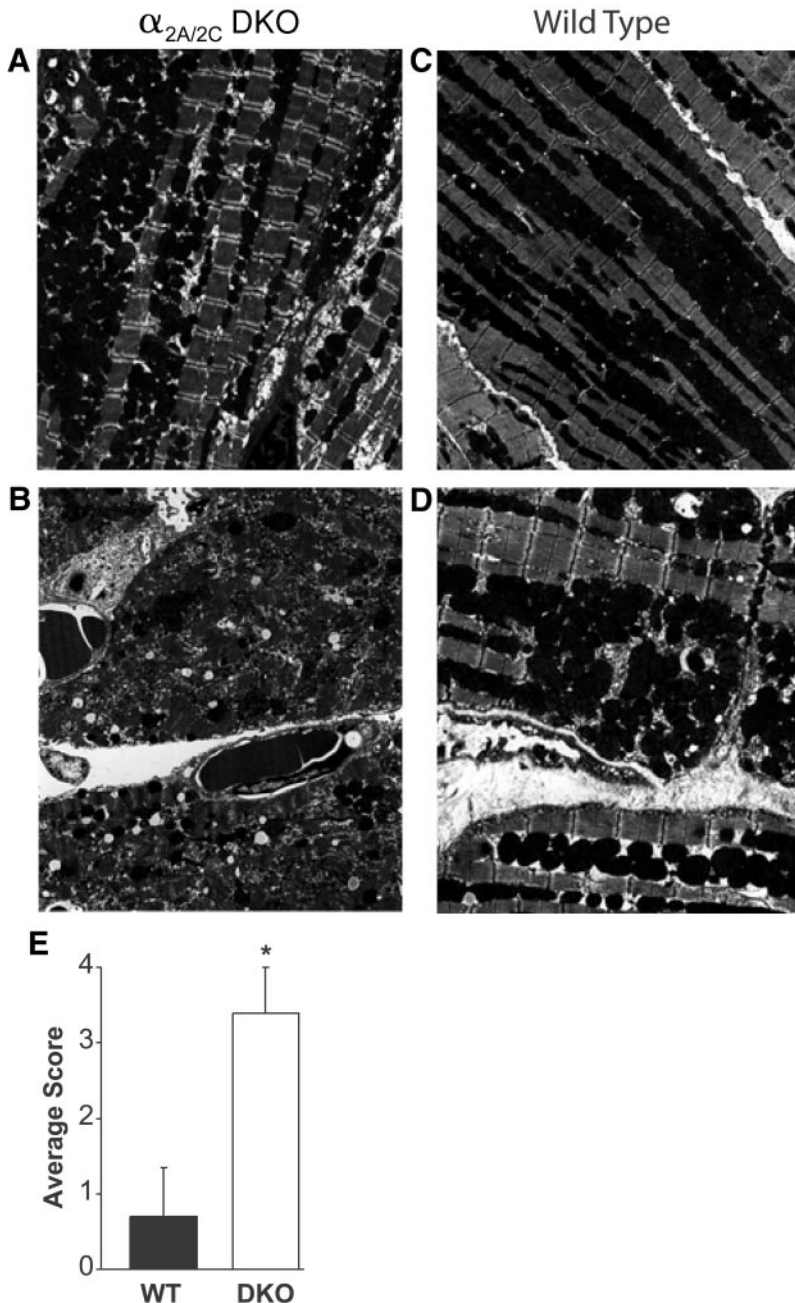


Fig. 5. Catecholamine-induced myocardial injury revealed by electron microscopy. Hearts from three DKO mice and two WT mice were fixed, sectioned, analyzed by a pathologist (J. Kosek) blinded to the genotype. Ten representative electron microscopy images were obtained from the left ventricle of each heart. Images were graded subjectively on a scale of 0–4, with 0 being normal and 4 being the most abnormal. Criteria used for grading included the integrity of the myofibrils, mitochondrial swelling or degeneration, and the appearance of vacuoles or fluid separating myofibrils and/or mitochondria. *A* and *B*: spectrum of histology observed in ventricles from DKO mice. *A* is the most normal micrograph and *B* is the most abnormal micrograph from the 30 images from 3 DKO mice. *C* and *D*: spectrum of histology observed in ventricles from WT mice. *C* is the most normal micrograph and *D* is the most abnormal micrograph from the 20 images from 2 WT mice. *E*: average injury score for WT and DKO mice. Sections of hearts from two WT and three DKO mice were scored for evidence of injury (range, 0–4; 0, least damage; 4, severe damage). The injury score was significantly higher in DKO mice compared with WT mice. The comparisons between WT and DKO mice were performed using Student's *t*-test. \*Significant difference between groups ( $P \leq 0.05$ ).

$\alpha_2$ -AR agonists such as clonidine suppress sympathetic tone primarily through effects on central nervous system  $\alpha_{2A}$ -ARs and have been used in the treatment of hypertension. A small clinical study (31) has suggested that clonidine may be beneficial in the treatment of heart failure. However, the beneficial effects of clonidine are limited by sedation, also mediated by the  $\alpha_{2A}$ -AR (22). Thus a selective  $\alpha_{2C}$ -AR agonist may provide sufficient control over catecholamine release to be beneficial in the prevention of heart failure without undesirable effects such as hypotension and sedation.

In conclusion,  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice develop functional and structural evidence of cardiac dysfunction by 4 mo of age. In contrast to most existing murine

models of heart failure, abnormal cardiac function in  $\alpha_{2A}/\alpha_{2C}$ -ARKO mice can be attributed to prolonged elevation of sympathetic activity rather than to genetic modifications that directly alter the expression of structural or functional proteins in the heart. These mice will provide a model system for better understanding the mechanism by which elevated sympathetic tone alone leads to deterioration in heart function. Moreover, these mice will be useful for both evaluating new pharmacological and genetic approaches for the prevention and treatment of heart failure.

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## REFERENCES

- Altman JD, Trendelenburg AU, MacMillan L, Bernstein D, Limbird L, Starke K, Kobilka BK, and Hein L. Abnormal regulation of the sympathetic nervous system in  $\alpha_{2A}$ -adrenergic receptor knockout mice. *Mol Pharmacol* 56: 154–161, 1999.
- Arber S, Hunter JJ, Ross J Jr, Hongo M, Sansig G, Borg J, Perriard JC, Chien KR, and Caroni P. MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. *Cell* 88: 393–403, 1997.
- Benjaminovitz A and Mancini DM. The role of exercise-based prognosticating algorithms in the selection of patients for heart transplantation. *Curr Opin Cardiol* 14: 114–120, 1999.
- Bond RA. Is paradoxical pharmacology a strategy worth pursuing? *Trends Pharmacol Sci* 22: 273–276, 2001.
- Bristow MR. The adrenergic nervous system in heart failure. *N Engl J Med* 311: 850–851, 1984.
- Bristow MR.  $\beta$ -Adrenergic receptor blockade in chronic heart failure. *Circulation* 101: 558–569, 2000.
- Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, and Jamieson S.  $\beta_1$ - and  $\beta_2$ -adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective  $\beta_1$ -receptor down-regulation in heart failure. *Circ Res* 59: 297–309, 1986.
- Chien KR. Stress pathways and heart failure. *Cell* 98: 555–558, 1999.
- Colucci WS. The effects of norepinephrine on myocardial biology: implications for the therapy of heart failure. *Clin Cardiol* 21: 120–124, 1998.
- D'Angelo DD, Sakata Y, Lorenz JN, Boivin GP, Walsh RA, Liggett SB, and Dorn GW 2nd. Transgenic  $G_{\alpha_q}$  overexpression induces cardiac contractile failure in mice. *Proc Natl Acad Sci USA* 94: 8121–8126, 1997.
- Desai KH, Sato R, Schauble E, Barsh GS, Kobilka BK, and Bernstein D. Cardiovascular indexes in the mouse at rest and with exercise: new tools to study models of cardiac disease. *Am J Physiol Heart Circ Physiol* 272: H1053–H1061, 1997.
- Engelhardt S, Hein L, Wiesmann F, and Lohse MJ. Progressive hypertrophy and heart failure in  $\beta_1$ -adrenergic receptor transgenic mice. *Proc Natl Acad Sci USA* 96: 7059–7064, 1999.
- Fentzke RC, Korcarz CE, Lang RM, Lin H, and Leiden JM. Dilated cardiomyopathy in transgenic mice expressing a dominant-negative CREB transcription factor in the heart. *J Clin Invest* 101: 2415–2426, 1998.
- Flesch M, Schiffer F, Zolk O, Pinto Y, Rosenkranz S, Hirth-Dietrich C, Arnold G, Paul M, and Bohm M. Contractile systolic and diastolic dysfunction in renin-induced hypertensive cardiomyopathy. *Hypertension* 30: 383–391, 1997.
- Haghighi K, Schmidt AG, Hoit BD, Brittsan AG, Yatani A, Lester JW, Zhai J, Kimura Y, Dorn GW 2nd, MacLennan DH, and Kranias EG. Superinhibition of sarcoplasmic reticulum function by phospholamban induces cardiac contractile failure. *J Biol Chem* 276: 24145–24152, 2001.
- Hajjar RJ, Muller FU, Schmitz W, Schnabel P, and Bohm M. Molecular aspects of adrenergic signal transduction in cardiac failure. *J Mol Med* 76: 747–755, 1998.
- Harding VB, Jones LR, Lefkowitz RJ, Koch WJ, and Rockman HA. Cardiac  $\beta$ ARK1 inhibition prolongs survival and augments  $\beta$  blocker therapy in a mouse model of severe heart failure. *Proc Natl Acad Sci USA* 98: 5809–5814, 2001.
- Hein L, Altman JD, and Kobilka BK. Two functionally distinct  $\alpha_2$ -adrenergic receptors regulate sympathetic neurotransmission. *Nature* 402: 181–184, 1999.
- Johns C, Gavras I, Handy DE, Salomao A, and Gavras H. Models of experimental hypertension in mice. *Hypertension* 28: 1064–1069, 1996.
- Kubota T, Bounoutas GS, Miyagishima M, Kadokami T, Sanders VJ, Bruton C, Robbins PD, McTiernan CF, and Feldman AM. Soluble tumor necrosis factor receptor abrogates myocardial inflammation but not hypertrophy in cytokine-induced cardiomyopathy. *Circulation* 101: 2518–2525, 2000.
- Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, and Feldman AM. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- $\alpha$ . *Circ Res* 81: 627–635, 1997.
- Lakhlani PP, MacMillan LB, Guo TZ, McCool BA, Lovinger DM, Maze M, and Limbird LE. Substitution of a mutant  $\alpha_{2A}$ -adrenergic receptor via “hit and run” gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo. *Proc Natl Acad Sci USA* 94: 9950–9955, 1997.
- Lefkowitz RJ, Rockman HA, and Koch WJ. Catecholamines, cardiac  $\beta$ -adrenergic receptors, and heart failure. *Circulation* 101: 1634–1637, 2000.
- Levine TB, Francis GS, Goldsmith SR, Simon AB, and Cohn JN. Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. *Am J Cardiol* 49: 1659–1666, 1982.
- Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, Roberts R, and Marian AJ. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. *Circulation* 103: 789–791, 2001.
- Limbird LE and Vaughan DE. Augmenting  $\beta$  receptors in the heart: short-term gains offset by long-term pains? *Proc Natl Acad Sci USA* 96: 7125–7127, 1999.
- Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D, Barsh GS, and Kobilka BK. Cardiovascular regulation in mice lacking  $\alpha_2$ -adrenergic receptor subtypes b and c. *Science* 273: 803–805, 1996.
- Link RE, Stevens MS, Kulatunga M, Scheinin M, Barsh GS, and Kobilka BK. Targeted inactivation of the gene encoding the mouse  $\alpha_{2c}$ -adrenoceptor homolog. *Mol Pharmacol* 48: 48–55, 1995.
- MacMillan LB, Hein L, Smith MS, Piascik MT, and Limbird LE. Central hypotensive effects of the  $\alpha_{2A}$ -adrenergic receptor subtype. *Science* 273: 801–803, 1996.
- Mancini DM, Eisen H, Kussmaul W, Mull R, Edmunds LH Jr, and Wilson JR. Value of peak exercise oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure. *Circulation* 83: 778–786, 1991.
- Manolis AJ, Olympios C, Sifaki M, Smirnioudis N, Handanis S, Argirakis S, Katsaros C, Gavras I, and Gavras H. Chronic sympathetic suppression in the treatment of chronic congestive heart failure. *Clin Exp Hypertens* 20: 717–731, 1998.
- Miller LW. Listing criteria for cardiac transplantation: results of an American Society of Transplant Physicians-National Institutes of Health conference. *Transplantation* 66: 947–951, 1998.
- Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, and Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93: 215–228, 1998.
- Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. *J Am Coll Cardiol* 20: 248–254, 1992.
- Post SR, Hammond HK, and Insel PA. Beta-adrenergic receptors and receptor signaling in heart failure. *Annu Rev Pharmacol Toxicol* 39: 343–360, 1999.
- Rohrer DK, Schauble EH, Desai KH, Kobilka BK, and Bernstein D. Alterations in dynamic heart rate control in the  $\beta_1$ -adrenergic receptor knockout mouse. *Am J Physiol Heart Circ Physiol* 274: H1184–H1193, 1998.
- Roth DM, Gao MH, Lai NC, Drumm J, Dalton N, Zhou JY, Zhu J, Entrikin D, and Hammond HK. Cardiac-directed adenylyl cyclase expression improves heart function in murine cardiomyopathy. *Circulation* 99: 3099–3102, 1999.
- Rothermel BA, McKinsey TA, Vega RB, Nicol RL, Mammen P, Yang J, Antos CL, Shelton JM, Bassel-Duby R, Olson EN, and Williams RS. Myocyte-enriched calcineurin-interacting protein, MCIP1, inhibits cardiac hypertrophy in vivo. *Proc Natl Acad Sci USA* 98: 3328–3333, 2001.

39. **Sharma S, Firoozi S, and McKenna WJ.** Value of exercise testing in assessing clinical state and prognosis in hypertrophic cardiomyopathy. *Cardiol Rev* 9: 70–76, 2001.
40. **Ungerer M, Bohm M, Elce JS, Erdmann E, and Lohse MJ.** Altered expression of  $\beta$ -adrenergic receptor kinase and  $\beta_1$ -adrenergic receptors in the failing human heart. *Circulation* 87: 454–463, 1993.
41. **Vatner DE, Asai K, Iwase M, Ishikawa Y, Shannon RP, Homcy CJ, and Vatner SF.**  $\beta$ -Adrenergic receptor-G protein-adenylyl cyclase signal transduction in the failing heart. *Am J Cardiol* 83: 80H–85H, 1999.
42. **Vikstrom KL, Factor SM, and Leinwand LA.** A murine model for hypertrophic cardiomyopathy. *Z Kardiol* 84: 49–54, 1995.
43. **Zhang SJ, Cheng H, Zhou YY, Wang DJ, Zhu W, Ziman B, Spurgoen H, Lefkowitz RJ, Lakatta EG, Koch WJ, and Xiao RP.** Inhibition of spontaneous  $\beta_2$ -adrenergic activation rescues  $\beta_1$ -adrenergic contractile response in cardiomyocytes overexpressing  $\beta_2$ -adrenoceptor. *J Biol Chem* 275: 21773–21779, 2000.

