Disruption of type 5 adenylyl cyclase prevents β-adrenergic receptor cardiomyopathy: A novel approach to β-adrenergic receptor blockade

Lin Yan, Stephen F. Vatner, and Dorothy E. Vatner

Departments of Cell Biology and Molecular Medicine and Medicine and the Cardiovascular Research Institute, Rutgers University-New Jersey Medical School, Newark, New Jersey

Submitted 14 July 2014; accepted in final form 3 September 2014

Yan L, Vatner SF, Vatner DE. Disruption of type 5 adenylyl cyclase prevents β-adrenergic receptor cardiomyopathy: A novel approach to β-adrenergic receptor blockade. Am J Physiol Heart Circ Physiol 307: H1521–H1528, 2014. First published September 5, 2014; doi:10.1152/ajpheart.00491.2014.—β-Adrenergic receptor (β-AR) blockade is widely used to treat heart failure, since the adverse effects of chronic β-AR stimulation are central to the pathogenesis of this disease state. Transgenic (Tg) mice, where β-AR signaling is chronically enhanced by overexpression of cardiac β2-ARs, is a surrogate for this mechanism, since these mice develop cardiomyopathy as reflected by reduced left ventricular (LV) function, increased fibrosis, apoptosis, and myocyte hypertrophy. We hypothesized that disruption of type 5 adenylyl cyclase (AC5), which is in the β-AR signaling pathway in the heart, but exerts only a minor β-AR blocking effect, could prevent the cardiomyopathy in β2-AR Tg mice without the negative effects of full β-AR blockade. Accordingly, we mated β2-AR Tg mice with AC5 knock out (KO) mice. The β2-AR Tg × AC5 KO bigenic mice prevented the cardiomyopathy as reflected by improved LV ejection fraction, reduced apoptosis, fibrosis, and myocyte size and preserved exercise capacity. The rescue was not simply due to a β-blocking effect of AC5 KO, since neither baseline LV function nor the response to isoproterenol was diminished substantially compared with the negative inotropic effects of β-blockade. However, AC5 disruption in β2-AR Tg activates the antioxidant, manganese superoxide dismutase, an important mechanism protecting the heart from cardiomyopathy. These results indicate that disruption of AC5 prevents the cardiomyopathy induced by chronically enhanced β-AR signaling in mice with overexpressed β2-AR, potentially by enhancing resistance to oxidative stress and apoptosis, suggesting a novel, alternative approach to β-AR blockade.

β-adrenergic receptor signaling: oxidative stress

MATERIALS AND METHODS

Animal models. The development and characterization of mice with cardiac-specific overexpression of the β2-AR used in this study have been described previously (13, 44). Parent β2-AR mice were obtained from Jackson Laboratories. Parent AC5 KO mice used in this study have been described previously (48, 64). The mice with cardiac-specific overexpression of the β2-AR were mated with AC5 KO mice to generate littermate wild-type (WT), β2-AR, AC5 KO, and β2-AR × AC5 KO bigenic mice. Four groups of animals were studied (WT, AC5 KO, β2-AR Tg, and β2-AR Tg × AC5 KO bigenic mice) at 13–16 mo of age. All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee at the New Jersey Medical School. All of the investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Echocardiography. Transthoracic echocardiography was performed in 13- to 16-mo age-matched mice using an Acuson Sequoia with a 13-MHz transducer. Mice were anesthetized with 2.5% tribromoethanol (Avertin) injected intraperitoneally and placed on a warmed saline bag. Electrocardiographic leads were attached to each limb using needle electrodes (Grass Technologies). After a short-axis...
two-dimensional (2-D) image of the left ventricle (LV) was obtained at the level of the papillary muscles, a 2-D guided M-mode tracing crossing the anterior and posterior wall was recorded at a sweep speed of 200 mm/s. The following parameters were measured on the M-mode tracings using the leading-edge technique: LV internal dimensions of diastole and systole (LVIDd, LVIDs), LV external dimensions of diastole and systole, and wall thickness at diastole and systole. LV ejection fraction (LVEF) was calculated by the cubed method: LVEF = [(LVIDd)^3 − (LVIDs)^3]/(LVIDd)^3.

Exercise capacity. Mice were exercised on the treadmill. All mice were subjected to a practice trial 3 days before the experiment to adapt to the treadmill testing environment. Food was withdrawn at least 3 h before the exercise. At the time of the experiment, each mouse was placed on a treadmill at a constant 10° angle. The treadmill was started at 4 m/min, and the speed incrementally increased 2 m/min every 2 min until the mice reached exhaustion. Exhaustion was defined as spending time (10 s) on the electric stimulus platform without attempting to reengage the treadmill belt. The maximal running distance was calculated based on the time and speed of running.

Isoproterenol challenge. Echocardiography was performed in WT and AC5 KO mice anesthetized with 2.5% tribromoethanol (290 mg/kg). For acute injection of isoproterenol (ISO), a PE-10 catheter was inserted in the right jugular vein, ISO was injected at the rate of 0.04 μg·kg\(^{-1}\)·min\(^{-1}\) for 5 min, and LVEF fraction was measured. In WT mice, after recovery, 0.5 mg/kg propranolol was administered intravenously followed by LVEF measurement. ISO was given again 3 min after propranolol was infused followed by LVEF measurement.

Western blot analysis. Total proteins were extracted from the LV of hearts as previously described (28, 64). Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Immunoblotting was performed using polyclonal antibodies to MnSOD from Millipore and cleaved caspase-3 from Cell Signaling. Immunodetection was accomplished using a donkey anti-rabbit secondary antibody (1:2,000 dilution) and the enhanced chemiluminescence kit (Amer sham Biosciences). Expression of these proteins was quantified by densitometry, and the data are presented as arbitrary units of density. GAPDH antibody Western blotting was used to verify equal protein loading of the blots.

Superoxide dismutase activity. The superoxide dismutase (SOD) activity was measured by an SOD Assay kit from Cayman Chemicals (Ann Arbor, MI) following the manufacturer’s instructions.

Histoogy. The heart was excised and washed in cold PBS. A ring of LV tissue, cut at the level of the papillary muscles, was fixed in 10% buffered formalin, processed, and embedded in paraffin. Sections were cut 6-μm thick and deparaffinized. Images were obtained using an Olympus microscope (Olympus BX 51) with a ×40 objective lens. Collagen volume percent was assessed using samples stained with picric sirius red. Myocyte cross-sectional area was determined on sections stained with rhodamine-labeled wheat germ agglutinin (1: 250; Vector). Apoptosis was determined by using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling, which detects apoptosis-induced DNA fragmentation by nick-end labeling of the fragmented DNA at the 3′-hydroxyl ends (Terminal Transferase, recombinant kit; Roche Diagnostics). The nuclei were costained with 4′,6-diamino-2-phenylindole. These techniques have all been used extensively (36, 50–52, 64). To further discriminate apoptosis in myocytes from smaller nonmyocytes, tissue sections (5 μm thickness) were costained with α-actinin for myocytes as previously described (18, 50, 51).

Statistical analysis. All data were expressed as means ± SE. To compare two independent groups, we used Student’s unpaired t-test. For a comparison of three or more groups, one-way ANOVA with Newman-Keuls post hoc test was used. P < 0.05 was taken as a minimal level of significance.

RESULTS

Cardiomyopathy in β2-AR Tg mice. β2-AR Tg mice compared with WT developed cardiomyopathy as evidenced by decreased (P < 0.05) baseline LVEF (53 ± 3 vs. 63 ± 1%) (Fig. 1A), fractional shortening (FS) (23 ± 1 vs. 28 ± 1%), as well as increased LV end-diastolic and end-systolic diameter (Table 1). In addition, LV weight-to-tibial length (wt/TL) ratio, an index of LV hypertrophy, and lung wt/TL ratio, an index of LV decompensation, were also elevated in old β1-AR Tg (Table 1). Maximal running distance during treadmill exercise was significantly reduced in β2-AR Tg mice compared with WT mice (Fig. 1B). Furthermore, histological evidence of cardiomyopathy was also present, as reflected by increased levels, compared with WT, of myocardial fibrosis (18.1 ± 2.6 vs. 2.1 ± 0.9%, P < 0.05) and LV hypertrophy, as assessed by myocyte cross-sectional area (756 ± 57 vs. 477 ± 35 mm\(^2\)) (Fig. 1, C and D). Apoptosis was also increased in the heart, P < 0.05, which occurred predominantly in nonmyocytes, and to a much lesser extent, in myocytes (Fig. 2A). Importantly, baseline levels of all these measurements in old AC5 KO were not different from WT, as exemplified by LVEF, which was 70 ± 2%, similar to the values in old WT shown above.

Mating β2-AR Tg with AC5 KO mice prevents the cardiomyopathy in β2-AR Tg mice. The cardiomyopathy was prevented in age-matched bigenic mice (β2-AR Tg × AC5 KO), as reflected by normal LVEF (70 ± 1%) and FS (33 ± 1%) (Fig. 1A). Increased LV end-diastolic and end-systolic diameter, as well as LV wt/TL ratio and Lung wt/TL ratio, were diminished in bigenic β2-AR Tg × AC5 KO mice (Table 1). Furthermore, apoptosis and fibrosis in the bigenic mice were no longer different from WT (Fig. 1, C and D, and Fig. 2A and B). Reduced exercise capacity was also restored in the β2-AR Tg × AC5 KO bigenic mice (Fig. 1B).

The prevention of cardiomyopathy by AC5 disruption is not simply due to β-AR blockade. Baseline LVEF was not decreased in AC5 KO mice compared with WT mice, whereas β-AR blockade with propranolol treatment reduced baseline LVEF significantly (P < 0.05, Fig. 3). The response to ISO challenge increased LVEF in WT and AC5 KO mice similarly, whereas β-AR blockade with propranolol fully abolished the increase in LVEF (Fig. 3). These data confirm that AC5 KO is not a β-blocker, and the prevention of cardiomyopathy by AC5 disruption is not simply due to β-AR blockade.

Protection of cardiomyopathy in β2-AR Tg mice by inhibition of oxidative stress. A major mechanism mediating the cardiac apoptosis and necrosis in cardiomyopathy is increased oxidative stress, and SOD is a major protective mechanism for oxidative stress. SOD activity and MnSOD levels were reduced in β2-AR Tg mice. Furthermore, apoptosis and fibrosis in the bigenic mice were no longer different from WT (Fig. 1, C and D, and Fig. 2A and B). Reduced exercise capacity was also restored in the β2-AR Tg × AC5 KO bigenic mice (Fig. 1B).

DISCUSSION

We demonstrated in this study that AC5 inhibition prevents the cardiomyopathy induced by chronic β-adrenergic receptor stimulation. The cardiomyopathy that developed as the mice aged was characterized by reduced cardiac function, increased cardiac apoptosis, fibrosis, and myocyte cross-sectional area, with reduced exercise tolerance. Except for reduced exercise...
tolerance, which has not been noted previously, prior studies also demonstrated the development of cardiomyopathy and increased mortality in β2-AR Tg mice (13, 52). Mortality was not examined in the current study because many mice had to be killed for the histological and biochemical studies, which reduced the power and statistical validity of a mortality analysis. A prior study indicated that the cause of the increased mortality in β2-AR Tg mice was due primarily to heart failure and potentially to arrhythmias, since ectopic beats were observed on the ECG (13). It is likely that the AC5 KO also protects against arrhythmias, since AC5 Tg mice are more susceptible to arrhythmias than AC5 KO or WT (65).

The potentially most clinically attractive feature of this prevention is that the older AC5 KO do not have reduced LV...
function at baseline, so adding AC5 inhibition to heart failure should not have the potential to reduce cardiac function further, yet the AC5 KO prevention of the adverse effects of chronic β-adrenergic receptor stimulation is still observed. One might predict that reducing AC activity will, by itself, be responsible for rescuing β-AR cardiomyopathy. However, the main source of AC activity in the heart is AC6, not AC5. We have demonstrated that AC5 KO reduces total AC activity in the heart by only 25–30% (30, 47). Our data in this study showing source of AC activity in the heart is AC6, not AC5. We have concluded that prevention of oxidative stress in the AC5 KO was augmented in cardiomyopathy develops, and these Tg mice are less tolerant to oxidative stress, whereas fibrosis and apoptosis were normalized in β2-AR Tg × AC5 KO bigenic mice, and MnSOD was augmented in β2-AR Tg × AC5 KO mice, supporting the conclusion that prevention of oxidative stress in the AC5 KO mice plays a role in preventing the cardiomyopathy in β2-AR Tg. Moreover, we previously demonstrated that upregulation of MnSOD and resistance to oxidative stress in the AC5 KO heart are mediated by activation of MEK/ERK and SIRT1/FoxO3a pathways (36, 64). It is conceivable that MEK/ERK and SIRT1/FoxO3a-mediated activation of MnSOD is also involved in prevention of cardiomyopathy developed in β2 Tg mice.

Interestingly, the predominant fraction of the increased apoptosis in the cardiomyopathy and the decrease, when the cardiomyopathy was prevented by the AC5 KO mechanism, occurred in nonmyocytes. Whereas this might be understood, recognizing that over 70% of cells in the heart are nonmyocytes, it seems inconsistent with the currently held concept that apoptosis leads to cardiac dysfunction through reducing myocyte numbers (4, 19). However, the current results are consistent with our previous findings that the apoptosis occurs predominantly in nonmyocytes in several models of cardiomyopathy, including ischemic cardiomyopathy (50) and hypertrophic cardiomyopathy (18, 51). Based on our previous studies, the cell types of apoptotic nonmyocytes mainly include macrophages, neutrophils, fibroblasts, and endothelial cells (50, 51). The role of nonmyocyte apoptosis in the heart in mediating or preventing the cardiomyopathy is not clear, since the nonmyocytes are a double-edged sword. On the one hand, many of these cell types have been linked to myocyte cell damage (1, 17, 18, 33, 41, 42, 50), whereas on the other hand they have been linked to rescue (25, 38, 58, 61). For example, activation of both macrophages or myofibroblasts plays a beneficial role in tissue repair and the improvement of cardiac remodeling and function after myocardial infarction (38, 62).

Exercise intolerance is not only uniformly observed in patients with heart failure but is generally an early sign of cardiac dysfunction that precedes evidence of reduced baseline function at rest (2, 5, 26, 37, 53). However, exercise capacity is rarely examined in mouse models of cardiomyopathy, and has not been examined in transgenic mice, where β-AR signaling is chronically enhanced. However, it is known that, when β-AR signaling is enhanced in young mice, either genetically by increasing components of the β-AR signaling pathway (16) or pharmacologically (45), exercise performance is improved. AC5 KO mice exhibit enhanced exercise performance, whereas the older β2-AR Tg mice that developed cardiomyopathy had depressed exercise capacity, which was rescued in the bigenic mice. This finding is important because
simply rescuing baseline cardiac function in patients with cardiomyopathy does not necessarily indicate that exercise tolerance will be normalized, leading to an improved quality of life.

Our current study supports the concept that inhibition of AC5 may be a novel approach to the treatment of cardiomyopathy/heart failure. β2-Blockers are well-established drugs for treating heart failure (43a). However, because β-blockers can cause contractile dysfunction (15, 27, 54), some patients are intolerant to the administration of β-blockers (7, 29, 35), and the number of such patients who cannot benefit from β-blockers is, roughly estimated, to be over 1.3 million (43, 56). Inhibition of AC5 may be superior to inhibition of β-AR stimulation, since it does not decrease LV contractility (47–49, 64), and may lead to more effective β-AR desensitization (49). To demonstrate this point, we compared the extent to which the response to β-AR stimulation was blocked either by the AC5 KO or by a β-AR blocker propranolol. AC5 KO reduced the response to isoproterenol minimally, whereas propranolol completely abolished it. Thus, the development of a pharmacological AC5 inhibitor could be a useful alternative to β-blockers.

Fig. 2. AC5 disruption prevents apoptosis in β2-AR cardiomyopathy predominantly in nonmyocytes, and to a much lesser extent, in myocytes. A: quantification of terminal deoxyribonucleotide transferase-mediated dUTP nick end-labeling (TUNEL) assay in LV sections of old mice showed that nonmyocyte apoptosis was significantly rescued in β2 Tg × AC5 KO bigenic mice, n = 4 / each group. B: representative images of the staining used to discriminate apoptosis in myocytes (bottom) from nonmyocytes (top). Myocytes were stained with sarcomeric α-actinin (red) and counterstained with TUNEL (green) and 4',6-diamino-2-phenylindole (DAPI, blue). The original magnification of the images was ×40. C: the levels of cleaved caspase 3 were significantly higher in β2 Tg hearts compared with AC5 KO. In contrast to the β2 Tg mice, the bigenic mice were no longer different from WT; n = 4 / group. Results are expressed as means ± SE. *P < 0.05 by one-way ANOVA. WT and AC5 KO values were not significantly different.
In summary, AC5 inhibition prevented the cardiomyopathy and depressed exercise tolerance induced by chronic β-adrenergic receptor stimulation. The prevention of cardiomyopathy by AC5 inhibition is not simply due, as might be thought, to reduced AC activity, thereby inhibiting sympathetic stimulation, as occurs with β-AR blockade, since AC activity is regulated to a minor extent by AC5 in the heart and consequently sympathetic stimulation was not blocked in the AC5 KO as it was with propranolol. Therefore, it is likely that there are distal mechanisms mediating the prevention, e.g., activation of SOD, which enhances resistance to oxidative stress through AC5 inhibition. Thus, AC5 inhibition may be a useful adjunct to β-AR blockade as a treatment for heart failure with the advantage of less inhibition of contractility.

ACKNOWLEDGMENTS

We thank Drs. Chunbo Wang, Shumin Gao, Grace Lee, Yimin Tian, Hui Ge, Chujun Yuan, and Ronald Pachon for providing support.

GRANTS

This study was supported by National Institutes of Health Grants 5P01-AG-027211, 5R21-H110052, 1R01-HL-102472, 5R01-HL-033107, 5T32-H110052, 5R01-HL-095888, 5P01-HL-069020, 5R01-HL-091781, R01-HL-106511, R01-HL-093481, and 1R01-HL-119464.

DISCLOSURES

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Author contributions: L.Y. performed experiments; L.Y. analyzed data; L.Y., S.F.V., and D.E.V. interpreted results of experiments; L.Y. prepared figures; L.Y. and S.F.V. drafted manuscript; L.Y., S.F.V., and D.E.V. edited and revised manuscript; S.F.V. and D.E.V. conception and design of research; S.F.V. and D.E.V. approved final version of manuscript.

Fig. 3. The effect of AC5 KO is not simply due to β-blockade. There is no decrease in baseline LVEF in AC5 KO mice, whereas β-blocker, propranolol, treatment reduced baseline LVEF. The response to isoproterenol (ISO) challenge is similar in WT (n = 6) and AC5KO (n = 4) mice. In contrast, the increase in LVEF with ISO is fully abolished by propranolol (n = 5). *P < 0.05 vs. WT by Student’s t-test analysis.

Fig. 4. Manganese superoxide dismutase (MnSOD) is activated in β2-AR Tg × AC5 KO bigenic mice. MnSOD expression levels determined by Western blotting (A) and superoxide dismutase (SOD) activity (B) were significantly downregulated in β2-AR Tg mice but preserved in β2-AR Tg × AC5 KO bigenic mice compared with WT mice; n = 4–7/group. Results are expressed as means ± SE. *P < 0.05 by one-way ANOVA. MnSOD activity was increased significantly, P <0.05, in AC5 KO mice.
REFERENCES


