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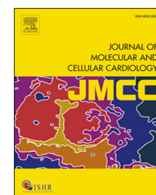

Original article

Deletion of the β2-adrenergic receptor prevents the development of cardiomyopathy in mice

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ABSTRACT

Beta adrenergic receptor (β-AR) subtypes act through diverse signaling cascades to modulate cardiac function and remodeling. Previous in vitro studies suggest that β1-AR signaling is cardiotoxic whereas β2-AR signaling is cardioprotective, and may be the case during ischemia/reperfusion in vivo. The objective of this study was to assess whether β2-ARs also played a cardioprotective role in the pathogenesis of non-ischemic forms of cardiomyopathy. To dissect the role of β1 vs β2-ARs in modulating MLP (Muscle LIM Protein) cardiomyopathy, we crossbred MLP −/− with β1 −/− or β2 −/− mice. Deletion of the β2-AR improved survival, cardiac function, exercise capacity and myocyte shortening; by contrast haploinsufficency of the β1-AR reduced survival. Pathologic changes in Ca^{2+} handling were reversed in the absence of β2-ARs: peak Ca^{2+} and SR Ca^{2+} were decreased in MLP −/− and β1 +/−/MLP −/− but restored in β2 −/− MLP −/−. These changes were associated with reversal of alterations in troponin I and phospholamban phosphorylation. Gi inhibition increased peak and baseline Ca^{2+}, recapitulating changes observed in the β2 −/−/MLP −/−. The L-type Ca^{2+} blocker verapamil significantly decreased cardiac function in β2 −/− MLP −/− vs WT. We next tested if the protective effects of β2-AR ablation were unique to the MLP model using TAC-induced heart failure. Similar to MLP, β2 −/− mice demonstrated delayed progression of heart failure with restoration of myocyte shortening and peak Ca^{2+} and Ca^{2+} release. Deletion of β2-ARs prevents the development of MLP −/− cardiomyopathy via positive modulation of Ca^{2+} due to removal of inhibitory Gi signaling and increased phosphorylation of troponin I and phospholamban. Similar effects were seen after TAC. Unlike previous models where β2-ARs were found to be cardioprotective, in these two models, β2-AR signaling appears to be deleterious, potentially through negative regulation of Ca^{2+} dynamics.

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1. Introduction

Beta adrenergic receptors (β-ARs) play a major role in the regulation of cardiac function. Their activation provides positive inotropic, chronotropic and lusitropic effects, however, β-ARs also play an important role in cardiac remodeling, and thus in the pathogenesis of dilated cardiomyopathy and heart failure. The continuous interaction between the underlying myocardial contractile dysfunction and the compensatory neurohumoral mechanisms activated by that dysfunction results in activation of β-AR signaling pathways that contribute to the progression of disease [1]. Of the two main β-AR subtypes in the heart (β1 and β2), β1-AR signaling is coupled to the stimulatory guanylyl nucleotide binding protein, Gs, leading to activation of adenylyl cyclase, increases in cAMP, activation of PKA and subsequent phosphorylation of key regulators of excitation–contraction coupling. β1-AR signaling has also been linked to cardiotoxic and pro-apoptotic signaling [2,3]. By contrast, β2-ARs not only signal through Gs but also through the inhibitory G protein, Gi, which attenuates the positive inotropic and chronotropic effects of β1-stimulation and activates additional signaling pathways involved in cardioprotection [4]. Thus, some have proposed that the β1-AR is the "cardiotoxic subtype" whereas the β2-AR is the "cardioprotective subtype." However, much of this data has been derived from in vitro studies in isolated cardiomyocytes, often with non-physiologic overexpression of the specific β-AR subtype being studied. Whether these in vitro studies will translate into in vivo models of heart failure is still unclear, although there is some in vivo data suggesting that the β2-AR is cardioprotective [5–8]. Still, the precise role of each β-AR subtype in the pathogenesis of cardiomyopathy and heart failure remains to be determined. These studies are crucial to designing the best therapeutic approach to β-AR modulation, as some have suggested that a combination of a β1-AR antagonist and a β2-AR agonist would result in a more favorable modulation of the β-AR system than the use of a non-subtype specific β-blocker alone [5].

One of the best described in vivo models of a genetic, non-ischemic cardiomyopathy is the Muscle LIM Protein (MLP) knockout mouse. MLP or cysteine-rich protein 3, contains two zinc finger LIM domains

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each followed by a glycine rich domain and it is known to interact with the titin-binding proteins α-actinin and T-cap at the Z-disc and β1-spectrin and the nebulin-related protein N-RAP at costameres and intercalated discs, respectively [9]. Mice deficient in MLP exhibit chamber dilation and contractile dysfunction, characteristics of dilated cardiomyopathy and transition to failure. This model is clinically relevant, as downregulation of MLP has been observed in patients with chronic heart failure [10] and mutations in the MLP gene have been identified in patients with dilated cardiomyopathy [11,12].

Previous studies have shown that MLP cardiomyopathy can be altered by changing components of the β-AR signaling system or its downstream effectors, although the exact mechanisms have yet to be worked out. Overexpression of the β2-AR did not rescue MLP cardiomyopathy, whereas overexpression of the GRK2 inhibitor, βARKct, did [13]. Ablation of phospholamban (PLB), an inhibitor of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), also rescued MLP mice, suggesting that defects in SR Ca^{2+} cycling play a pivotal role in progression towards heart failure in this model [14]. Although alterations in Ca^{2+} transients were described associated with this rescue, the mechanisms were largely undefined. In the present study, we assessed the role of β1 vs β2-AR signaling in modulating MLP cardiomyopathy and heart failure. Contrary to expectations based on other models, we found that deletion of the β2-AR rescued and deletion of the β1-AR worsened MLP cardiomyopathy, suggesting that β2-AR signaling was playing a deleterious role and β1-AR signaling a cardioprotective role. We further determined a mechanism by which β2-AR deletion restores myocyte shortening in MLP mice, through improving Ca^{2+} availability. To further assess if the cardioprotection provided by ablation of the β2-AR was unique to the MLP model we assessed the effects of β2-AR deletion in a model of transverse aortic constriction (TAC)-induced heart failure and confirmed that absence of β2-ARs also attenuated the progression of heart failure and restored Ca^{2+} dynamics.

2. Materials and methods

A more detailed version of materials and methods is included in Supplementary methods.

2.1. Generation of β-AR/MLP knockouts

Crosses were carried out between homozygous β1−/− and β2−/− mice (FVB background) generated by our lab [15,16] and homozygous MLP−/− mice (FVB/Sv129), kindly provided by Dr. Ken Chien. WT littermate controls were used to ensure comparability between the different lines. β2−/−MLP−/− were generated by crossing MLP−/− with β2−/− mice which produced F1 heterozygous MLP+/− and β2+/−; these were then crossed to generate F2 double knockouts. The same approach was used to generate β1−/−MLP−/−, however due to the near total in utero mortality of the homozygous double knockouts, only β1+/−/MLP−/− were used for further studies. Mice were genotyped by PCR to confirm β1-AR, β2-AR and MLP disruptions. All procedures were approved by the Stanford Administrative Panel on Laboratory Animal Care.

2.2. Transverse aortic constriction-induced heart failure

Heart failure was induced by TAC as previously reported [17]. TAC was performed in C57BL/6J and β2−/− in C57BL/6J background as we have previously described [18]. Echocardiography was performed before surgery and 1, 2 and 4 weeks after TAC. Sham-operated controls consisted of age-matched mice that underwent an identical surgical procedure including isolation of the aortic arch, but without banding.

2.3. Echocardiography

Images were acquired with a GE Vivid 7 ultrasound system (GE health care, Milwaukee, WI) equipped with a 10 MHz transducer. Baseline measurements included left ventricular internal dimension at end-diastole (LVIDd) and left ventricular internal dimension in systole (LVIDs). Left ventricular fractional shortening (%FS) was calculated.

2.4. Incremental treadmill exercise

Baseline metabolic measurements during exercise were performed utilizing a Simplex II metabolic rodent treadmill (Columbus Instruments, Columbus, OH) as previously described [19].

2.5. Isolation of left ventricular myocytes

Adult ventricular myocytes were isolated from 6 month old mice based on previously published protocols [20,21] with modifications. Experiments were performed with freshly isolated myocytes resuspended in a HEPES-buffered solution (in mM 1 $CaCl_2$, 137 NaCl, 5.4 KCl, 15 dextrose, 1.3 $MgSO_4$, 1.2 NaH_2PO_4, 20 HEPES, pH 7.4).

2.6. Myocyte shortening and relengthening

Cell contraction properties of myocytes were evaluated with a video-based sarcomere spacing acquisition system (SarcLen, IonOptix, Milton, MA) as previously described [22,23]. Changes in sarcomere length were recorded and analyzed using IonWizard software (IonOptix, Milton, MA).

2.7. Ca^{2+} transient measurements

A separate set of myocytes was loaded with 0.5 μM fura 2-acetoxymethyl ester (Molecular Probes, Eugene, Oregon) for 15 min. Cells were excited at 340 and 380 nm, continuously alternated, at rates as high as 250 pairs/s using a HyperSwitch system (IonOptix, Milton, MA). Background-corrected fura 2 ratios were collected at 510 nm. This ratio is independent of cell geometry and excitation light intensity, and reflects the intracellular Ca^{2+} concentration [24,25].

2.8. Sarcoplasmic reticulum Ca^{2+} measurements

Caffeine 10 mM was used to induce Ca^{2+} release from the SR; maximum fluorescence was used as a measure of SR Ca^{2+}, as previously described [26].

2.9. Gi protein inhibition

1.5 μg/ml pertussis toxin (PTX) (Enzo Life Sciences, Plymouth Meeting, PA) was administered to freshly isolated WT myocytes for 3 h to inhibit Gi as previously described [27]. Ca^{2+} transient measurements were then performed after PTX treatment.

2.10. Immunoblotting

Mouse hearts were homogenized; proteins were quantified and probed against SERCA2 ATPase, PLB (PLB), phospho-CaM Kinase II Thr286, calsequestrin (CSQ) (Affinity BioReagents, Rockford, IL), phospho-PLB Ser16 (Millipore, Billerica, MA), phospho-PLB Thr17 (Badrilla, Leeds, United Kingdom), Na^{+}/ Ca^{2+} exchanger-1 (NCX) (Abcam) CaMKII, troponin (TnI), phosho-TnI Ser23-24 (Cell Signaling Technology, Danvers, MA) and ryanodine receptor (RyR), phospho RyR Ser2809 and phospho RyR Ser2815 (a kind gift of Dr. Andrew Marks, Columbia University).

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