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β-Blockers Restore Calcium Release Channel Function and Improve Cardiac Muscle Performance in Human Heart Failure

Steven Reiken, PhD; Xander H.T. Wehrens, MD, PhD; John A. Vest, MD; Alessandro Barbone, MD; Stefan Klotz, MD; Donna Mancini, MD; Daniel Burkhoff, MD; Andrew R. Marks, MD

Background—Chronic β-adrenergic receptor (β-AR) blockade improves cardiac contractility and prolongs survival in patients with heart failure; however, the mechanisms underlying these favorable responses are poorly understood. Stress-induced activation of the sympathetic nervous system results in protein kinase A (PKA)-mediated phosphorylation of the calcium (Ca^{2+}) release channel/cardiac ryanodine receptor (RyR2), required for cardiac excitation-contraction (EC) coupling, activating the RyR2 channel, and increasing cardiac contractility. The hyperadrenergic state of heart failure results in leaky RyR2 channels attributable to PKA hyperphosphorylation and depletion of the stabilizing FK506 binding protein, FKBP12.6. We tested the hypothesis that improved cardiac muscle function attributable to β-AR blockade is associated with restoration of normal RyR2 channel function in patients with heart failure.

Methods and Results—We assessed the effects of β-AR blockade on left ventricular volume using isolated perfused hearts and β-agonist responsiveness using muscle strips from patients undergoing transplantation. Twenty-four human hearts were examined, 10 from patients with heart failure treated with β-AR blockers (carvedilol, metoprolol, or atenolol), 9 from patients with heart failure without β-AR blocker treatment, and 5 normal hearts. RyR2 PKA phosphorylation was determined by back-phosphorylation, FKBP12.6 in the RyR2 macromolecular complex was determined by coimmunoprecipitation, and channel function was assayed using planar lipid bilayers. β-AR blockers reduced left ventricular volume (reverse remodeling) and restored β-agonist response in cardiac muscle from patients with heart failure. Improved cardiac muscle function was associated with restoration of normal FKBP12.6 levels in the RyR2 macromolecular complex and RyR2 channel function.

Conclusions—Improved cardiac muscle function during β-AR blockade is associated with improved cardiac Ca^{2+} release channel function in patients with heart failure. **(*Circulation*. 2003;107:2459-2466.)**

Key Words: heart failure ■ calcium ■ ion channels ■ remodeling ■ catecholamines

Although β-adrenergic receptor (β-AR) blocker therapy improves survival in patients with heart failure,[1–3] the mechanism by which this class of drugs improves cardiac function in humans has not been determined. The rationale for using β-ARs in patients with heart failure is that the sympathetic nervous system is hyperactive in these individuals.[4] It is not, however, intuitively obvious how blocking a pathway that increases contractility in normal hearts should be therapeutic in patients with decreased cardiac function. Indeed, the use of β-AR blockers in heart failure is counterintuitive, because acute administration of this class of drugs decreases contractility in normal and failing hearts. Perhaps because of this contradiction, the use of β-AR blockade in patients with heart failure is less widespread than it should be based on large clinical trials showing improved survival in all classes of patients with heart failure. Better understanding of the mechanisms underlying the favorable effects of long-term β-AR blockade in patients with heart failure should lead to more appropriate use of this therapy and should help with the identification of novel therapeutic targets.

See p 2395

It is well established that ligand binding to the β-AR activates adenylyl cyclase via G-proteins, resulting in elevated cAMP levels and protein kinase A (PKA) activation. Moreover, β-ARs are downregulated in failing hearts and uncoupled from downstream signaling via G proteins. It is presumed that cAMP is decreased and PKA activity reduced in cardiomyocytes from failing hearts.[5] However, only a few studies have examined the PKA phosphorylation of substrates other than phospholamban in failing hearts.[6]

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The cardiac ryanodine receptor (RyR2)/calcium (Ca^{2+}) release channel that regulates cardiac excitation-contraction (EC) coupling is a macromolecular complex that includes PKA and its targeting protein mAKAP.[7] RyR2 is PKA hyperphosphorylated in failing hearts,[6] resulting in dissociation of the regulatory subunit, the FK506 binding protein, FKBP12.6. FKBP12.6 depletion from the RyR2 macromolecular complex yields channels that are pathologically hypersensitive to Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR).[6] In a canine model of rapid pacing-induced heart failure, these alterations in RyR2 structure and function are restored to normal by treatment with the β_1-AR selective blocker metoprolol.[8]

In the present study, we show that chronic systemic β-AR blockade can induce reverse remodeling in human failing hearts,[9] as evidenced by a significant reduction in left ventricular (LV) volume and improved contractile response to exogenous β-adrenergic stimulation. Moreover, this reverse remodeling is associated with normalization of the stoichiometry of the RyR2 macromolecular complex and channel function that may in part explain some of the beneficial effects of β-AR blockade in patients with severe heart failure.

Methods

Patient Demographics, Medical History, and Baseline Cardiac Function

Patients' information was obtained from medical records, pretransplant evaluations, and electronic medical record database. Ejection fractions determined from echocardiograms, left ventriculograms, and gated equilibrium radionuclide ventriculography scans and heart rates were obtained immediately before cardiac transplantation. Medical regimens were obtained from inpatient charts at the time of transplant and outpatient medical records. Medications listed were those taken immediately before transplant. Patients assigned to the β-blocker group were identified by outpatient records and pretransplant evaluations as having been treated chronically with β-blockers before transplant.

Heart Harvest and Pressure Volume Relationships

Data were from 19 human hearts of patients with end-stage heart failure after orthotopic heart transplant under a protocol approved by the Institutional Review Board of the New York Presbyterian Hospital. Ten of the patients received β-blockers before transplant as part of their medical treatments, and 9 did not. Data were also obtained from 5 normal hearts not suitable for transplantation. Hearts were preserved with cold (4°C) hypocalcemic, hyperkalemic cardioplegia solution at explant. Passive ventricular pressure-volume relationships were measured by placing compliant balloons in the right ventricle (RV) and left ventricle (LV), as detailed previously.[10,11] Chamber sizes were indexed by the volume, yielding an intraventricular pressure of 30 mm Hg (LVV_{30} and RVV_{30}, respectively). Data are expressed as mean±SD. Comparisons between groups were performed with unpaired *t* tests; for comparisons of more than 2 groups, ANOVA was used. $P<0.05$ was considered statistically significant.

Myocardial Force Generation in Response to β-Adrenergic Stimulation

Baseline force generation and response to β-adrenergic stimulation were measured from trabeculae (<1 mm diameter) isolated from the LV in a subset of patients, as described previously.[9,11]

β-Adrenergic Receptor Density

β-antagonist binding studies were performed to determine β-receptor density in right and left ventricular myocardium, as previously described.[8]

Immunoprecipitation and Back-Phosphorylation of Ryanodine Receptor

Myocardial homogenates were prepared, RyR2 was immunoprecipitated, and PKA phosphorylation was determined as described previously.[6]

RyR2 Phosphospecific Antibody (RyR2-P2809)

Rabbit polyclonal antibody was raised against a KLH-conjugated peptide based on the human RyR2 sequence phosphorylated at Ser^{2809} (CRTRRIS$^{(PO4)}$QTSQV), with a cysteine added to the N-terminus. The anti-sera were affinity purified using the antigenic peptide. The purified antibody (anti–RyR2-2809P) specifically recognizes RyR2 PKA phosphorylated on Ser^{2809} and does not react with dephosphorylated RyR2. Samples containing human cardiac SR (25 μg) from normal and failing hearts as well as canine cardiac SR (10 μg) phosphorylated with PKA (40 U in phosphorylation buffer[6]) in the presence and absence of PKI (500 nmol/L) were size fractionated on 6% SDS-PAGE. PKA-phosphorylated RyR2 was determined by probing the immunoblots with the anti–RyR2-2809P (1:5000 dilution), whereas total RyR2 protein was measured using a previously described antibody[6] that recognizes the C-terminus of the channel (anti–RyR-5029, 1:3000).

RyR2 Single-Channel Recordings

Single-channel recordings of RyR2 were performed and analyzed under voltage-clamp conditions, as described previously.[12,13]

Results

Patient demographics, hemodynamics at the time of transplant, and baseline cardiac function data of β-blocker treatment group (heart failure patients receiving β-blockers before transplant) and of a control group (transplant patients not receiving β-blockers) are summarized in Table 1. There were more males in the β-blocker group. Heart rate was lower in the β-blocker treatment group (91±13 versus 73±11 beats/min in controls [n=8] versus β-blocker [n=8], $P<0.05$). Because cardiac output was similar in both groups, we infer that stroke volume was elevated in the β-blocker group. Otherwise, no differences in demographics, hemodynamics at the time of transplant, or baseline cardiac function between the β-blocker treatment group and the control group were statistically significant. The demographics of the subset of patients (n=8 for the β-blocker treatment group and n=9 for the control group) in whom hemodynamic data were measured were similar to those of the total study population. The patients' medical regimens at the time of transplant are summarized in Table 2. Only medications relevant to the treatment of heart failure are listed. Hemodynamic data were not available for the patients with normal hearts.

Diastolic Pressure-Volume Relationships

Compared with control patients, LV end-diastolic pressure-volume relationships (EDPVR) of transplant patients not receiving β-AR blockers were shifted rightward toward markedly elevated volumes (Figure 1A). For patients receiving β-AR blockers, the LV EDPVR was shifted toward lower volumes, although not reaching that of control patients. Thus, despite similar hemodynamics, the hearts of patients receiv-

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TABLE 1. Patient Demographics, Hemodynamics, and Cardiac Function

	Heart Failure	Heart Failure + β-Blocker
Demographics		
No. patients	9	10
Age, y	49±13	49±14
Male/female, n	5/4	7/3
DCM/ICM, n	6/3	6/4
Hemodynamics at time of transplantation	(n=9)	(n=8)
Central venous pressure, mm Hg	10±5	12±8
Pulmonary artery diastolic pressure, mm Hg	20±7	18±6
Mean pulmonary artery pressure, mm Hg	27±10	25±10
Mean arterial pressure, mm Hg	73±9	81±14
Cardiac output, L/min	3.3±0.9	3.7±1.1
Heart rate, bpm	91±13 (n=8)	73±11*
Cardiac function		
Ejection fraction, %	19±3 (n=9)	20±6 (n=10)
$\dot{V}o_2$ max, mL/kg per min	14±3 (n=7)	13±4 (n=8)

Values are given as mean ± SD or number of patients. DCM indicates dilated cardiomyopathy; ICM, ischemic cardiomyopathy.

*$P<0.05$ compared with heart failure.

ing β-AR blockers were smaller, suggesting that β-AR blockers induced structural reverse remodeling. Similar observations were made for RV. As summarized in Figure 1B, the volume at a filling pressure of 30 mm Hg (V_{30}), which is an index of ventricular size, for both ventricles was reduced in the β-AR blocker group.

Myocardial Force Generation in Response to β-Adrenergic Stimulation

LV myocardial trabeculae were obtained fresh as the hearts were explanted and were superfused in a muscle bath. The physical characteristics and resting tension at L_{max} of these trabeculae did not differ significantly between heart failure and β-AR blocker–treated transplant patients. Baseline force was slightly lower in the patients treated with β-blockers (17.3±15.7 versus 14.4±5.8 versus 9.1±3 mN/mm^2 for the heart failure group [n=9], β-blocker group [n=8], and control group [n=5], respectively, P=NS). On exposure to 1 μmol/L isoproterenol, absolute force increased to slightly higher levels in the group treated with β-blockers (23.62±17.3 versus 25.87±7.2 versus 24±10.4 mN/mm^2 for the heart failure group [n=9], β-blocker group [n=8], and control group [n=5], respectively, P=NS), so that the percent increase in force after exposure to isoproterenol was significantly higher in the β-blocker group (Figure 2).

Parameters characterizing dynamics of contraction at baseline and after exposure to 1 μmol/L isoproterenol were available from a subset of the muscles studied. Isoproterenol increased the rates of contraction and relaxation in all groups.

TABLE 2. Medical Regimens

	Heart Failure (n=9)		Heart Failure + β-Blocker (n=10)	
	n	Average Daily Dose, mg/day	n	Average Daily Dose, mg/day
β-Blocker	0		10	
Atenolol	0	0	1	25
Carvedilol	0	0	8	21.4
Metoprolol	0	0	1	50
Diuretic	9		10	
Furosemide	8	257	9	162
Metolazone	3	2.5	0	0
Torsemide	1	100	1	40
Spironolactone	2	28	4	17.5
ACE inhibitor/ARB	6		9	
Captopril	2	43.75	2	84.4
Enalapril	1	5	2	17.5
Fosinopril	0	0	1	30
Lisinopril	2	15	4	12.5
Losartan	1	50	0	0
Digoxin	9	0.172	7	0.339
Inotrope	7		5	
Dobutamine	6	...	3	...
Milrinone	5	...	3	...

ACE indicates angiotensin II–converting enzyme; ARB, angiotensin II receptor blocker.

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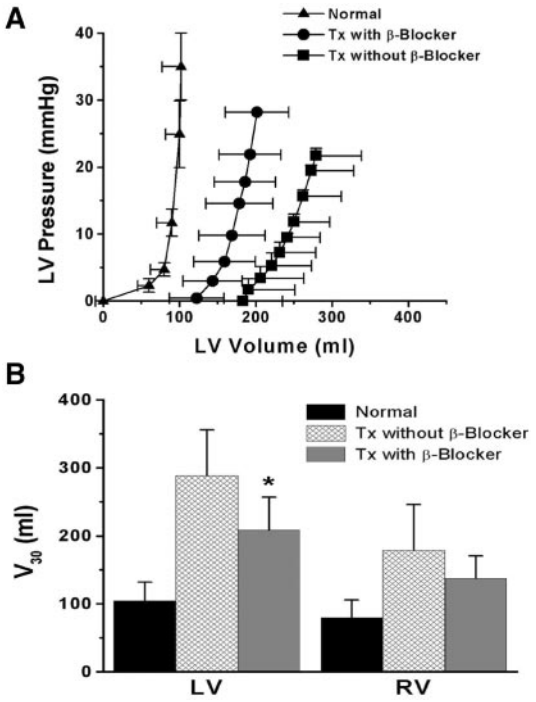

Figure 1. A, Ex vivo, passive pressure-volume relationships measured from hearts of transplant patients receiving β-blockers (circles: Tx with β-blockers, n=6) were shifted toward significantly lower volumes with steeper slope compared with those measured from transplant patients not receiving β-blockers (squares: Tx without β-blockers, n=5). These curves were shifted toward larger volumes with lower slopes than those of normal hearts not suitable for transplant (triangles: normal, n=5). B, Volume at which ex vivo passive pressure is 30 mm Hg (V_{30}) for the LV and RV of hearts from the 3 different groups of hearts (normal, n=5; treatment without β-blocker, n=7; treatment with β-blocker, n=6). *$P<0.05$ for therapy with β-AR blocker (Tx with β-blocker) vs therapy without β-AR blocker (Tx without β-blocker).

There was a trend toward an enhancement of these effects of isoproterenol in patients treated with β-blockers, as shown by a greater decrease in the duration of contraction after exposure to isoproterenol in the β-blocker group (702±50.1 versus 488±83.1 ms, n=3) compared with heart failure patients not treated with β-blockers (787±218 versus 638±175 ms, n=7, $P<0.05$).

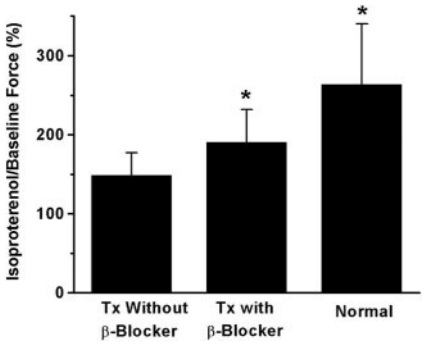

Figure 2. Isometric peak force (expressed as a percent of baseline force) attained during isoproterenol stimulation (1 μmol/L) from left ventricular trabeculae of control patients, transplant patients, and transplant patients treated with β-blockers. *$P<0.05$ for therapy with β-AR blocker (Tx with β-blocker, n=8) vs therapy without β-AR blocker (Tx without β-blocker, n=9) and for normal hearts (normal, n=5) vs therapy without β-AR blocker.

β-AR density was reduced in heart failure. In controls, the B_{max} was 50.0±2.8 fmol/mg and the K_d was 22.7±1.1 nmol/L (n=2) and was reduced to 23.1±3.4 fmol/mg and 13.3±1.4 nmol/L in heart failure patients (n=4, $P<0.001$). Treatment with β-blockers restored the β-AR density toward normal levels of 36.2±8.6 fmol/mg and 16.8±3.2 nmol/L (n=4, P=NS compared with controls). This finding is in contrast to previous studies, which have not shown an increase in β-AR density in patients treated with carvedilol,[14] indicating that at least in some patients the drug can share this effect with other β-AR blockers such as metoprolol.

PKA Hyperphosphorylation of the Cardiac Ryanodine Receptor

Consistent with our previous findings,[6] PKA phosphorylation of immunoprecipitated RyR2 assessed with back-phosphorylation was significantly increased in failing human hearts (Figure 3). β-AR blocker treatment restored PKA phosphorylation of RyR2 in failing hearts to the levels seen in nonfailing hearts (Figure 3). The stoichiometry of PKA phosphorylation of RyR2 from unfailing hearts was 0.8±0.14 moles of phosphate per mole of channel (n=2), compared with 2.8±0.2 moles of phosphate per mole of channel from failing hearts (n=5, $P<0.001$ compared with unfailing hearts) and 1.8±0.6 moles of phosphate per mole of channel (n=5, $P=0.013$ compared with failing hearts) from failing hearts in patients treated with β-AR blockers. In failing hearts, 3 of the 4 PKA sites on the tetrameric RyR2 were phosphorylated in vivo, whereas only 1 was PKA phosphorylated in unfailing hearts and 1 or 2 sites in failing hearts in patients treated with β-AR blockers. RyR2 PKA hyperphosphorylation in failing hearts was confirmed using a phosphoepitope-specific anti–RyR2-2809P antibody. The specificity of the anti–RyR2-2809P antibody was demonstrated by immunoblot analyses of PKA-phosphorylated RyR2 (Figure 3C). Phosphorylation of cardiac SR with PKA dramatically increased the PKA-phosphorylated RyR2 signal detected using the phosphoepitope-specific anti–RyR2-2809P antibody compared with the signal for PKA-phosphorylated RyR2 from non–PKA-treated SR. This increase in PKA-phosphorylated RyR2 signal was specifically inhibited by the PKA inhibitor PKI. Equal amounts of RyR2 were loaded in each sample, as demonstrated by immunoblots using the anti–RyR-5029 antibody that recognizes the extreme carboxy terminus of RyR2. The data obtained using the phosphoepitope-specific anti-RyR2-2809P antibody confirmed that RyR2 from failing human hearts was PKA hyperphosphorylated compared with RyR2 from control hearts and from β-blocker–treated patients (Figure 3C).

The RyR2 macromolecular complex includes RyR2, FKBP12.6, PKA, the protein phosphatases PP1 and PP2A, and their targeting proteins, mAKAP, spinophilin, and PR130, respectively.[7] The components of the macromolecular complex, RyR2, were assessed by coimmunoprecipitation from cardiac homogenates and immunoblotting (Figure 4A). There was a significant depletion of PP1, PP2A, and

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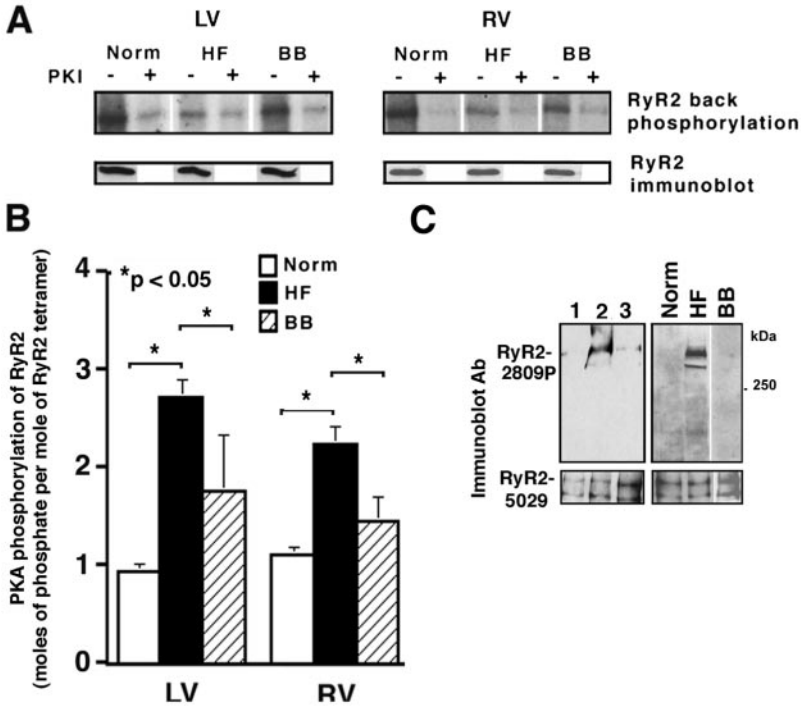

Figure 3. β-blockers reduce PKA-mediated phosphorylation of RyR2. PKA phosphorylation of RyR2 was measured in the LV and RV of normal human hearts (Norm), heart failure patients (HF), and heart failure patients treated with β-adrenergic receptor blockers (BB). A, Immunoprecipitated RyR2 was phosphorylated with PKA (5 U), and the PKA inhibitor (PKI_{5-24}) was used to demonstrate specificity of the phosphorylation. Equivalent amounts of RyR2 protein were used in each kinase reaction, as shown by immunoblotting. B, Relative PKA phosphorylation of RyR2 from heart homogenates from control patients (Norm, n=2), heart failure patients (HF, n=5), and heart failure patients treated with β-adrenergic receptor blockers (BB, n=5) were determined by dividing the specific phosphorylation signal by the amount of RyR2 protein (determined by immunoblotting and densitometry). Results expressed as inverse of PKA-dependent [γ-^{32}P] ATP signal±SD. C, Immunoblots probed for PKA phosphorylated RyR2 with phosphospecific antibody (anti-RyR2-2809P that recognizes RyR2 PKA phosphorylated on Ser^{2809} and total RyR2 with anti–RyR-5029). Lane 1 contains 10 μg of canine cardiac SR microsomes; lanes 2 and 3, 10 μg canine cardiac SR microsomes phosphorylated with PKA (40 units) in absence (lane 2) and presence of PKI (lane 3). Cardiac SR (25 μg) from normal (Norm), failing (HF), and failing human heart treated with β-blockers (BB), size fractionated using 6% PAGE and immunobloted with anti–RyR2-2809P and anti–RyR-5029.

FKBP12.6 in the RyR2 macromolecular complex in heart failure patients not receiving β-blockers (Figures 4A and 4B). In patients receiving β-AR blockers, the amounts of PP1, PP2A, and FKBP12.6 were restored to normal (Figures 4A and 4B). Changes in the amount of FKBP12.6 in the RyR2 macromolecular complex were not attributable to changes in total cellular FKBP12.6, because these levels did not change with heart failure or with β-blockers (Figure 4C).

Cardiac Ryanodine Receptor Channel Function

To assess the effect of β-AR blocker therapy on channel function, we examined the single channel properties of RyR2 in planar lipid bilayers.[6] Twenty channels from 2 nonfailing human hearts, 28 channels from 5 failing human hearts, and 34 channels from 5 failing hearts from patients treated with β-blockers were studied (Figure 5). None of the RyR2 channels from normal hearts exhibited significantly increased P_o or f_o, compared with 25 of 28 (89%) of the RyR2 channels from failing hearts that exhibited significantly increased P_o or f_o ($P<0.001$, Figure 5). Both increased P_o and increased f_o are seen when FKBP12.6 is removed from RyR2.[6,15] In contrast, for RyR2 channels from failing hearts from patients treated with β-blockers only, 2 of 34 (6%) showed increased P_o or f_o ($P<0.001$ compared with heart failure).

Discussion

The present study shows that systemic β-AR blockade upregulates β-receptor density and restores normal macromolecular complex composition and function to the RyR2 channel in human failing hearts. There was associated restoration of myocardial β-AR agonist response and reverse structural remodeling of the LV and RV. Taken together, these data suggest that one of the beneficial effects of β-AR blocker therapy may be to improve cardiac muscle function by reversing a maladaptive defect in Ca^{2+} signaling in cardiac myocytes in failing hearts.

Sympathetic nervous system activation results in PKA phosphorylation of RyR2 and activation of the channel.[6,7,16–25] We have previously shown that PKA hyperphosphorylation of RyR2 in failing hearts shifts the sensitivity of RyR2 to Ca^{2+}-induced Ca^{2+} release to the left,[6] resulting in leaky channels (channels with increased sensitivity to Ca^{2+}-induced Ca^{2+} release) that can cause a diastolic SR Ca^{2+} leak.

In failing hearts, PKA hyperphosphorylation of RyR2 is associated with depletion of the regulatory protein FKBP12.6 in the channel macromolecular complex.[6] RyR2 channels that are depleted of FKBP12.6 exhibit increased sensitivity to Ca^{2+}-induced activation[6] and reduced coupled gating.[26,27] RyR2 channels are homotetramers, and each subunit contains a single PKA phosphorylation site (Ser^{2809} and 1 molecule of FKBP12.6 is bound to each subunit).[6,15,28] Thus, for a single RyR2 channel, there are 4 PKA sites and 4 FKBP12.6. The activity of key molecules that regulate the Ca^{2+} signal, which drives cardiac contractility, are increased by PKA phosphorylation.[20] These include the L-type Ca^{2+} channel, which is the trigger for cardiac EC coupling,[29,30] RyR2, which is the Ca^{2+}-release channel, and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a, via phosphorylation of phospholamban), which is the SR Ca^{2+} uptake pump. This integrated physiological circuit provides a mechanism for increasing SR Ca^{2+} release by increasing the activity of the trigger, the release channel, and the reuptake pump. Additional refinement of this modulatory signaling pathway is provided by the ability to PKA phosphorylate 1, 2, 3, or 4 of the RyR2 subunits. As each subunit is phosphorylated, thereby dissociating 1 FKBP12.6 molecule from the channel,

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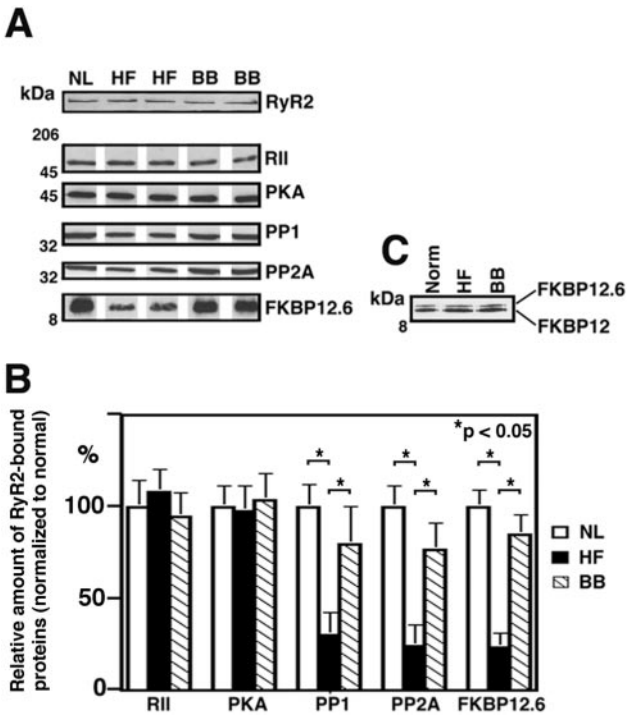

Figure 4. β-adrenergic receptor blockade normalizes levels of proteins in RyR2 macromolecular complex. Heart homogenates were immunoprecipitated with anti-RyR antibody. A, Representative immunoblots shown for components of RyR2 macromolecular complex, RyR2, PKA, RII, PP1 and PP2A, and FKBP12.6 using hearts from control patients (NL, n=2), heart failure patients (HF, n=5), and heart failure treated with β-blockers (BB, n=5). B, Protein levels were quantified using densitometry of immunoblots. Results expressed as relative amount of each component of RyR2 macromolecular complex corrected for the amount of RyR2 in each immunoprecipitation. Error bars are SD of the mean. C, Immunoblot showing equivalent amounts of total cellular FKBP12.6 in cardiac homogenates (20 μg) from normal hearts (NL), failing hearts (HF), and heart failure patients treated with β-blockers (BB).

the resulting shift to the left in the sensitivity to Ca^{2+}-induced activation of the channel causes a small but potentially significant increase in SR Ca^{2+} release, in part by increasing the sensitivity of RyR2 to activation by the Ca^{2+} that fluxes in via the L-type channel. This system therefore provides the possibility of a graded response to stress in which cardiac contractility can be modulated in response to metabolic requirements.

Heart failure is a new syndrome in evolutionary time, and as a post–reproductive age syndrome, it likely has never been subjected to evolutionary pressure. As such, the beautifully integrated physiological signaling pathway that provides graded increases in cardiac output in response to PKA phosphorylation of the key Ca^{2+} handling molecules may well become defective in failing hearts, representing a maladaptive response. In response to the inability of the weakened heart to increase cardiac output, the sympathetic nervous system remains chronically activated. This maladaptive response exacerbates heart failure in part by inducing a defect in SR Ca^{2+} release that additionally impairs contraction. These deficits are seen in both idiopathic cardiomyopathy (where there is global myocyte dysfunction) and in ischemic cardiomyopathy (where left ventricular dysfunction is initially attributable to a loss of myocytes), in which contractile function of otherwise normal myocytes becomes defective.[6] In addition, the PKA-hyperphosphorylated RyR2 may be the source of diastolic releases of SR Ca^{2+} that has been appreciated for years as being linked to delayed afterdepolarizations that are in turn felt to be the triggers for fatal ventricular arrhythmias.

β-AR blockade is one of the most effective treatments for heart failure. However, the use of β-AR blockers in patients with heart failure is counterintuitive, because they are known to decrease contractility acutely in normal and failing hearts. Systemic oral administration of β-AR blockers reverses PKA hyperphosphorylation of RyR2, restores the stoichiometry of the RyR2 macromolecular complex, and normalizes single-channel function in a canine model of heart failure.[8,31] The present study extends these observations to humans with heart failure and demonstrates that chronic β-AR blocker treatment can restore normal RyR2 complex composition and function. These data suggest that the counterintuitive effects of the β-AR blocker class of drugs in failing hearts may be explained by their ability to counteract the maladaptive response to the chronic hyperadrenergic state of heart failure described above and potentially restore the EC coupling machinery to a normal physiological mode. Although it is well established that β-blockers improve cardiac contractility in heart failure patients in large clinical trials, the present study was designed to assess the effects of β-blocker therapy on RyR2 channel function and cardiac muscle function.

In failing hearts, β-AR blockers would restore normal responsiveness to the system by resetting the PKA phosphorylation state of the RyR2 channel and restoring sensitivity to β-agonists, as we have shown in the present study. In addition, in patients with idiopathic dilated cardiomyopathy, it has recently been reported that treatment with β-AR blockers increases SERCA2a and α-myosin heavy chain mRNAs and a decrease in β-myosin heavy chain mRNA.[32] Taken together, these changes in RyR2 function and the upregulation of SERCA2a and α-myosin heavy chain likely contribute to improved cardiac contractility observed in patients treated with β-AR blockers.

The distinction between the acute and chronic effects of β-AR blockers may be explained by the finding that one of their effects is to restore not only the PKA phosphorylation state of the RyR2 channel but also the normal stoichiometry of the RyR2 macromolecular complex. This would in effect restore the RyR2 channel back to its normal physiological state and therefore could explain the positive effects of β-AR blockers on failing cardiac muscle as opposed to the negative effects on normal cardiac muscle, which is not PKA hyperphosphorylated to begin with. The present findings identify several novel potential therapeutic targets in the RyR2 macromolecular complex for the treatment of heart failure.

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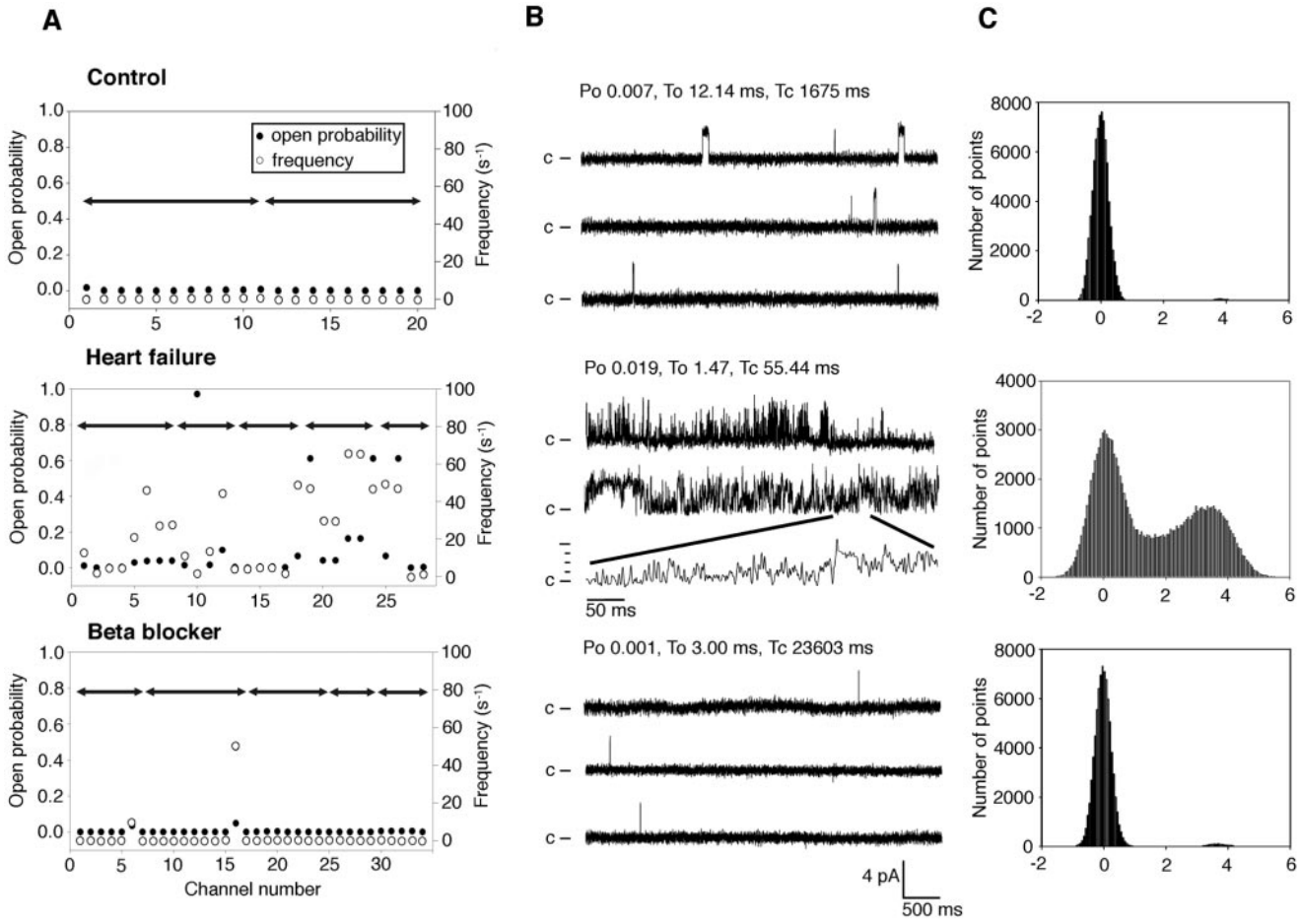

Figure 5. β-blockers normalize RyR2 function in failing human hearts. RyR2 channels from 3 groups, control, heart failure, and heart failure patients treated with β-blockers, were studied in planar lipid bilayers. A, Open probability (P_o, ●) and gating frequency (f_o, ○) shown for RyR2 channels. B, Representative single channel tracings from each group. A section of the single channel tracing from heart failure group (middle row) is shown with expanded time scale to demonstrate subconductance states (indicated by the markings to left of the tracing). C, Corresponding all-points amplitude histograms for each group. [Ca^{2+}] in the *cis* chamber was 150 nmol/L. Recordings were at 0 mV, closed state of channels are indicated (c), channel openings are upward, and horizontal lines indicate source of channels. *$P<0.05$ compared with heart failure.

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