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Arrhythmogenic Remodeling of β_2 versus β_1 Adrenergic Signaling in the Human Failing Heart

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Abstract

Background—Arrhythmia is the major cause of death in patients with heart failure, for which β -adrenergic receptor (AR) blockers are a mainstay therapy. But the role of β -adrenergic signaling in electrophysiology and arrhythmias has never been studied in human ventricles.

Methods and Results—We used optical imaging of action potentials (AP) and $[Ca^{2+}]_i$ transients (CaT) to compare the β_1 - and β_2 -adrenergic responses in left ventricular wedge preparations of human donor and failing hearts. β_1 -stimulation significantly increased conduction velocity (CV), shortened AP duration (APD), CaT duration (CaD) in donor but not failing hearts, due to desensitization of β_1 -AR in heart failure. In contrast, β_2 -stimulation increased CV in both donor and failing hearts but shortened APD only in failing hearts. β_2 -stimulation also affected transmural heterogeneity in APD but not in CaD. Both β_1 - and β_2 -stimulation augmented the vulnerability and frequency of ectopic activity and enhanced substrates for ventricular tachycardia in failing, but not donor, hearts. Both β_1 - and β_2 -stimulation enhanced Purkinje fiber automaticity, while only β_2 -stimulation promoted Ca-mediated premature ventricular contractions in heart failure.

Conclusions—During end-stage heart failure, β_2 -stimulation creates arrhythmogenic substrates via CV regulation and transmurally heterogeneous repolarization. β_2 -stimulation is, therefore, more arrhythmogenic than β_1 -stimulation. In particular, β_2 -stimulation increases the transmural difference between CaD and APD, which facilitates the formation of delayed afterdepolarizations.

Keywords

arrhythmia (mechanisms); heart failure; calcium; adrenergic receptor; delayed afterdepolarization; β_1 ; β_2 adrenergic receptor

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Introduction

Increased β -adrenergic sympathetic activity is a hallmark of heart failure and often leads to ventricular remodeling and impaired cardiac contractile function. Up to 50% of heart failure mortality is sudden and due to arrhythmias. β_1 or non-selective adrenergic blockers can reverse ventricular remodeling and reduce mortality in heart failure patients.¹ Although the antiarrhythmic effect of β -blockers has long been recognized,² the underlying mechanism is incompletely understood.³ So far, the effect of β -adrenergic signaling on electrophysiology and arrhythmias has never been studied in tissue preparations from failing and non-failing human hearts.

β -adrenergic stimulation can lead to sarcoplasmic reticulum (SR) Ca overload, spontaneous SR Ca release and delayed afterdepolarization (DAD).⁴ In addition, β -receptors regulate multiple ion channels via the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) cascade, which is electrogenic and potentially arrhythmogenic. However, cellular studies cannot fully explain arrhythmogenesis and arrhythmia maintenance at the tissue level. Potential arrhythmogenic substrates due to β -stimulation include abnormal impulse conduction, altered repolarization dynamics, and transmural heterogeneity in repolarization. The human ventricular free wall is comprised of three layers: subendocardium, midmyocardium, and subepicardium. These layers possess different intrinsic electrical properties that account for activation delays and synchronized ventricular repolarization.⁵ In heart failure, these properties are altered due to molecular and structural remodeling, including remodeling of β -adrenergic receptors (AR), and may set the stage for triggered arrhythmias, as seen in patients with heart failure.

Two dominant subtypes of β -AR are β_1 - and β_2 -AR. While β_1 -AR predominates in the human myocardium, β_2 -AR is equally important due to its more efficient adenylyl cyclase coupling and endogenous role in cardiac regulation.⁶ Recently, the use of non-selective β -blockers or β_2 -blockers was advocated by clinical trials and studies^{7, 8}, emphasizing the importance of β_2 -adrenergic signaling in heart failure. Although the effects of remodeling the β_1/β_2 -adrenergic system on contractility have been studied in human trabeculae,⁹ the effect β_1/β_2 -remodeling on electrophysiology and arrhythmogenesis in the left ventricular wall of the human failing heart is unknown.

We hypothesized that in end-stage heart failure, β_1 - and β_2 -adrenergic signaling are differentially remodeled and have different effects on arrhythmogenesis. To compare β_1 and β_2 selective adrenergic effects, we assessed EC coupling using dual optical mapping of AP and CaT in left ventricular wedge preparations from human failing (F) and donor (D) hearts. Our data indicate that β_2 -stimulation, in contrast to β_1 -stimulation, induces transmural heterogeneity in repolarization and sets the stage for arrhythmias. Furthermore, we show that β_2 -AR signaling enables the formation of DADs by increasing the difference between CaD and APD, suggesting an underlying mechanism of triggered arrhythmias, such as those observed in patients with heart failure.

Material and Methods

Experimental Protocol

Procurement and use of human myocardium was approved by the Institutional Review Board of Washington University School of Medicine. Non-ischemic end-stage failing human hearts (F, n=8) were procured at the time of transplantation at Barnes-Jewish Hospital. Nonfailing donor hearts (D, n=8) were procured at Mid-America Transplant Services (St. Louis, MO) and were used as controls. The clinical data of patients are shown in Supplemental Table 1.

The arterially perfused LV wedge preparations (Supplemental Figure 1) and dual optical mapping were described previously^{10, 11} and in Supplemental Methods. Fluorescent probes RH237 and Rhod-2AM were utilized for AP and CaT, respectively. We used selective β_2 -AR agonist Procaterol (1 μ mol/L)¹², selective β_1 -AR agonist Xamoterol (1 μ mol/L)¹³, and non-specific β -AR agonist Isoproterenol (ISO, 100nmol/L) (Tocris Bioscience, Ellisville, MO) (Supplemental Figure 2). The agonists were applied at saturating concentrations for maximum effective activation of the receptors^{12, 13}. Complete washout evaluated by the recovery of CV, APD and CaD was applied between Xamoterol and Procaterol.

Statistical Analysis

For studies in which baseline and β -stimulation measures were compared, we conducted analyses with a mixed random effects repeated measures model with group, time, and group-by-time interaction as fixed effects. For each analysis, several covariance structures were fit, and the fit with the smallest Bayesian Information Criteria score was used, which was the Toeplitz matrix structure. For experiments in which transmural layers were analyzed separately, we used a mixed random effects, split-plot study design to account for layers having different degrees of freedom relative to the group. Post-hoc t-tests were then carried out with Bonferroni correction. We considered $p < 0.05/(\text{number of pairwise tests})$ to be statistically significant.

In Supplemental Methods, we provide detailed data analysis and describe experimental protocols and methods, including Western blot, q-PCR, and immunofluorescence.

Results

Effects of β_2 - versus β_1 -AR stimulation on arrhythmia substrates in heart failure

Effects of β_2 -AR stimulation on CV—We quantified anisotropy as the ratio between longitudinal conduction velocity (CV_L) and transverse conduction velocity (CV_T)¹⁴ (Figure 1). In both donor and failing hearts, CV_T did not demonstrate a physiologically significant difference after β_1 - or β_2 -stimulation, though there was a statistically significant difference for β_2 -stimulation in failure (Figure 1C-D). Both β_1 and β_2 agonists dramatically increased CV_L in donor hearts. In failing hearts, only β_2 , but not β_1 , agonist increased CV_L (Figure 1E-F). The quantitative analysis of velocity changes are shown in Supplementary Figure 3. Accordingly, anisotropy decreased with β_1 -stimulation and increased with β_2 -stimulation in

failing hearts; however, in donor hearts conduction anisotropy is only increased significantly during β_2 -stimulation (Figure 1G-H).

β_2 -stimulation increased transmural heterogeneity of repolarization in failing hearts—To assess the effect of β -stimulation on repolarization we quantified APD at 80% of repolarization in the subendocardium (endo), midmyocardium (mid), and subepicardium (epi) (Figure 2). Classification of all layers is shown in Supplemental Figure 2. As previous studies have also shown¹⁵, we determined that APD in failing hearts, measured at different pacing rates in the absence or presence of β -stimulation, is significantly longer than APD in donor hearts (Figure 2B). Both β -agonists shortened APD in both groups. However, for the different layers, the agonists reduced APD to varying degrees (Figure 2C and Supplemental Table 2). This layer-dependent APD reduction increased the APD difference between endocardium and epicardium ($APD_{80endo}-APD_{80epi}$) dramatically more in failing hearts than in donor hearts (Figure 2D).

We quantified the layer-dependent APD reduction (ΔAPD) at 1Hz pacing in response to both β -agonists as the difference of APD values between baseline and in the presence of β -agonists (Figure 3A). ΔAPD maps revealed that β_1 - and β_2 -stimulation each resulted in different spatial patterns of APD reduction (Figure 3B).

In donor hearts, β_2 -agonist stimulation caused decreasing ΔAPD s (increasing reductions in APD) from the epicardium toward the endocardium. The opposite is seen in failing hearts, where β_2 -agonist treatment caused increasing ΔAPD s (decreasing reductions in APD) from the endocardium to the epicardium (Figure 3C). Taken together, the ΔAPD effect sizes between donor and failing hearts were greatest at the epicardium and decreased toward the endocardium. Strongly significant group-layer interactions for ΔAPD values ($p<0.0001$) highlight the differences in effect sizes between donor and failing hearts (Figure 3C). In failing hearts, the average APD reduction caused by β_2 -agonist in the transmural wall is greater compared with that in donor hearts. This suggests that the β_2 -AR pathway is sensitized in heart failure. β_1 -agonist treatment produced APD reductions that were most pronounced in the endocardium and decreased toward the epicardium of donor hearts. In failing hearts, a modest and uniform APD reduction was observed. In donor hearts, β_1 -agonist induced a significant APD reduction in all layers. This demonstrates that the β_1 -AR pathway is desensitized in failing hearts (Figure 3D).

We aimed to determine the molecular mechanism underlying the layer-dependency of β_2 -stimulation by quantifying β_2 -AR protein expression. In contrast with previous findings¹⁶, we observed that, in donor hearts, the expression of β_2 -ARs was higher in the endocardium compared with the epicardium, with a β_2 -AR-epi/ β_2 -AR-endo ratio less than one. In failing hearts, the expression of β_2 -ARs was reversed: higher expression is observed in the epicardium than the endocardium (Figure 3E) and a β_2 -AR-epi/ β_2 -AR-endo ratio greater than one. The reversed transmural gradients in β_2 -AR protein expression may explain the reversed endocardial-to-epicardial gradient effect of β_2 -stimulation on APD in failing hearts.

Phosphorylation of β_2 -AR by PKA was reduced in failing human hearts— β -stimulation controls electrophysiological targets primarily via the stimulatory G-proteins

(G_s) and downstream protein kinase A (PKA). Unlike β_1 -AR, which couples only to G_s, β_2 -AR can couple either to G_s or to inhibitory G-protein (G_i).¹⁵ Phosphorylation of β_2 -AR by PKA can switch its coupling from G_s to G_i.¹⁷ Therefore, we assessed β_2 -AR phosphorylation at PKA-phosphorylation site Ser345/Ser346¹⁸ and found β_2 -AR phosphorylation was significantly lower in failing compared with donor hearts (Figure 3F), suggesting greater coupling to G_s in failure.

Effect of β_2 -AR stimulation on CaD—We recorded CaT simultaneously with AP to generate CaD maps (Figure 4A-B). CaD values at 80% of Ca relaxation are shown in Supplemental Figure 4. CaD shortening (Δ CaD) was quantified in order to evaluate the effects of β -stimulation on Ca reuptake. β_1 -agonist treatment induced significantly greater CaD reductions in donor compared to failing hearts across the transmural wall (Figure 4D; the effect size between donor and failing hearts was transmurally homogeneous, as the group effect was strongly significant ($p = 0.0007$) while the group-layer interaction was not significant ($p = 0.66$). In contrast, β_2 -agonist treatment shortened CaD to a similar degree in both donor and failing hearts (Figure 4C). Interestingly, stimulation with the β_2 -agonist did not induce similar transmural spatial differences as those observed with APD reduction.

Transmural gradient of CaD-APD—The CaD-APD is a measure of the period during which the intracellular calcium concentration ($[Ca^{2+}]_i$) remains at a high level after the membrane has been completely repolarized (schematic in Figure 4E). Prolongation of this period, defined as Δ (CaD-APD) = (CaD-APD)_{stimulation} β - (CaD-APD)_{baseline}, could lead to a window where the $[Ca^{2+}]_i$ is higher after β -stimulation than at baseline (green arrows in Figure 4E) during the period of complete membrane repolarization. In this vulnerable window, DADs may occur due to inward currents from the Na-Ca exchanger (NCX) while it extrudes Ca^{2+} . In failing hearts, we observed prolongation of the Δ (CaD-APD) after β_2 -stimulation in the epicardium (-42.8 ± 10.7 ms for donor versus 13.6 ± 6.9 ms for failing, $p = 0.003$) and midmyocardium (-32.0 ± 10.1 ms for donor versus 7.1 ± 7.2 ms for failing, $p = 0.0074$) but not in the endocardium (-23.1 ± 8.0 ms for donor versus -31.6 ± 12.1 ms for failing, $p = 0.53$), where negative values indicates that CaD-APD value after stimulation is shorter than baseline condition. In donor hearts, prolongation of Δ (CaD-APD) after β_2 -stimulation was not observed (Figure 4F). Also, there were no significant differences in transmural Δ (CaD-APD)s in either donor or failing hearts after β_1 -stimulation (Figure 4G).

β_2 -AR stimulation altered CaT morphology—To evaluate changes in CaT morphology, we quantified the CaD₃₀/CaD₈₀ ratio.¹¹ We observed that the ratio is greater at baseline in failing hearts than in donor hearts throughout the transmural wall, as previous findings have also shown¹⁵ (Figure 5A). Treating with β_2 -agonist equalized the ratio (0.55 ± 0.02 versus 0.55 ± 0.01 , ns after β_2 -stimulation; and 0.52 ± 0.015 versus 0.59 ± 0.009 , $p = 0.003$ at baseline; donor vs. failing), which indicates a change in CaT morphology (Figure 5B and E). While the ratio in failing hearts was still greater than in donor hearts after treatment with the β_1 -agonist, transmural heterogeneity was eliminated in both groups (Figure 5C and F); this is demonstrated by a strongly significant group-layer interaction at baseline ($p < 0.0001$), but the absence of a significant group-layer interaction after treatment with β_1 -agonist ($p = 0.076$).

We analyzed Ca^{2+} release and extrusion to determine the period in which β_2 -stimulation causes changes in CaT morphology. We did not observe a difference in Ca^{2+} release when evaluating the rise time of CaT (Supplemental Figure 5). The Ca^{2+} extrusion was analyzed by determining two phases: the early phase (fast Ca^{2+} decline component), as determined by T_{50} (period of time from CaT peak to 50% reuptake), and the late phase (slow Ca^{2+} decline component), as determined by the Ca^{2+} decay constant. The T_{50} average for all layers decreased after β_2 -stimulation in both donor and failing hearts (Figure 5G). However, the Ca^{2+} decay constant was similar before and after β_2 -stimulation in failing hearts (Figure 5H), which suggests that abrogation of β_2 regulation of Ca^{2+} handling occurred mainly during the slow Ca^{2+} reuptake phase.

β -AR stimulation is necessary for ectopic activity in human heart failure—We counted premature ventricular contractions (PVCs) during 8 minutes of pacing (4 minutes of 0.5 and 4 minutes of 0.67 Hz) and automaticity after pacing. We did not observe spontaneous automaticity or PVCs under any condition in donor hearts. In contrast, failing hearts exhibited both ectopic PVCs and automaticity in response to β -stimulation. Multiple types of PVCs were observed after β_2 -stimulation. Figure 6A shows automaticity and PVCs with different morphologies originating from different locations recorded after β_2 -stimulation in failing hearts. PVC#1 represents a type of irregularly occurring PVC with small amplitude and slow upstroke that propagated through a limited area, as circled, before being terminated. According to the AP signal amplitude map, these PVCs originated from the midmyocardium. PVC#2 represents another type of PVCs with greater amplitude and fast upstroke that can propagate through the transmural surface. This type of PVC originated from the endocardium (Figure 6A).

Next, we analyzed the underlying mechanisms and involvement of calcium overload in these two types of PVCs (#1 and #2). We used AP-CaT delay-map to measure the time difference between AP activation and Ca release. Figure 6B shows a PVC#1 where the Ca release occurred prior to AP upstroke, which suggests that it was triggered by Ca release. Thus, the mechanism responsible for PVC#1 may be a Ca-driven process enhanced by β_2 -stimulation. In all hearts, Ca-driven PVCs only originated from the subepicardium and midmyocardium (Figure 7B), which were the transmural regions with prolonged CaD-APD.

PVC#2 shared the same origin with a series of regular beats of enhanced automaticity. This automaticity originated from the endocardium with a rate of 33 beats/min and was eliminated by rapid delivery of Lugol's solution to the subendocardial surface, suggesting that these events are due to the Purkinje fiber automaticity.¹⁹ To test this hypothesis, we determined the presence of the Purkinje fiber marker Cx40 by immunofluorescence.²⁰ Figure 6D top-right shows that the tissue from where the regular beats originated has high expression of Cx40 at the intercalated disk, further implicating that Purkinje fiber automaticity underlies the observed events.

β -AR stimulation increased occurrence of spontaneous PVCs and rapid pacing induced VT in failing hearts

To confirm the described mechanisms of automaticity and ectopy, we used real-time pseudo-ECG and monophasic AP recordings to measure the occurrences of PVCs and pacing induced VT (Figure 7A). The number of PVCs and VTs dramatically increased after β -AR stimulation in failing hearts, but neither could be induced in donor hearts (Figure 7C-E). Both β_1 - and β_2 -stimulation induced more automaticity and PVCs in the endocardium as compared to other regions, which mainly originated in the Purkinje fibers. Unlike β_1 -, which induced automaticity in the Purkinje fibers only, β_2 -agonist also induced PVCs in epicardium and midmyocardium in addition to endocardium, among which more than 44% were Ca-mediated. No Ca-driven PVCs were observed during β_1 -stimulation (Figure 7B).

Discussion

Our data show, for the first time, a differential role of β_1 - and β_2 -AR pathways in arrhythmogenesis in human heart failure. We show that in failing hearts, β_1 - and β_2 -stimulation provoke automaticity and PVCs through at least two mechanisms. Both β -ARs increase automaticity in Purkinje fibers, but only β_2 -AR stimulation causes Ca-driven PVCs. β_2 -stimulation further enhances arrhythmogenic substrates via CV regulation, spatially heterogeneous repolarization, and CaD. Importantly, β_2 -stimulation increased the transmural difference between CaD and APD, which facilitated the formation of DAD (Summarized in Figure 8B).

Arrhythmogenesis due to β_2 - and β_1 -stimulation during end-stage heart failure

Desensitization of β_1 -AR and preserved effects of β_2 -AR have been previously reported in gene/protein expression,¹⁶ and cardiac mechanics⁹ in human endocardial biopsies from patients with heart failure. We mapped the entire transmural LV and found sensitization of β_2 -AR at the subendocardium and midmyocardium of failing hearts. Moreover, in contrast to attenuated β_1 -regulation in failing hearts, β_2 -regulation is not only sensitized but also remodeled at the molecular and tissue level. This remodeling develops an arrhythmic substrate involving conduction, repolarization, and Ca handling in end-stage heart failure. Although β_2 -stimulation affected CV similarly in failing and donor hearts, the average anisotropy was increased only in failing hearts. Due to this increase, the impulse propagates slowly in the transverse direction but rapidly in the longitudinal direction, which may lead to conduction block²¹, while β_1 -stimulation lowers such vulnerability by decreasing the anisotropy. In addition, β_2 -stimulation (but not β_1 -stimulation) induced remodeling of repolarization in time- and space-domains such as altered refractoriness and increased transmural heterogeneity, which would widen the vulnerability window and facilitate unidirectional block and reentrant arrhythmias.²² The additional arrhythmogenicity resulting from β_2 -stimulation in the human failing myocardium provides mechanism for recent studies advocating the use of non-selective β -blockers or β_2 -specific blockers for heart failure.^{7, 8}

Switching of β_2 -AR coupling from G_i to G_s in failing hearts

β_2 -AR stimulation resulted in significant reduction of APD across the transmural surface in failing compared to donor hearts, which suggests sensitization of β_2 -AR in heart failure.

This effect may result from a β_2 -AR coupling switch from G_i to G_s proteins due to β_2 -AR phosphorylation by PKA, which is also activated by G_s .¹⁷ We observed significantly reduced β_2 -AR phosphorylation in failing hearts, which suggests that β_2 -ARs switch from G_i to G_s in heart failure. The switch from the protective G_i -dependent negative feedback control system to an unstable G_s -dependent positive feedback control system was rendered via remodeling of β_2 -AR signaling (Figure 8A) in heart failure. Therefore, β_2 -AR could enhance its control of EC coupling through switching from G_i to G_s , as evident from enhanced APD in failing hearts. Our observations are in agreement with studies by the group of Harding.²³ Their data showed a negative inotropic effect in failing human ventricular cardiomyocytes after application of β_2 -AR antagonist ICI118,551. In contrast, cardiomyocytes from non-failing hearts showed no significant decreased inotropic effect in the presence of β_2 -AR antagonist. These data further support our conclusion that, in heart failure, a greater proportion of β_2 -AR couples with G_s . Furthermore, β_2 -AR overexpression has a negative inotropic effect following treatment with ICI118,551 in mouse models.²⁴ An increased proportion of β_2 -AR- G_s coupling was reported in this transgenic mouse model,²⁴ which, along with previously described work in human hearts²³, lends support to our theory.

G_i to G_s switch abrogates increase of NCX activity caused by β_2 -stimulation

In this study, we established that abrogation of β_2 regulation during the late phase of Ca extrusion accounted for CaT morphological change in heart failure. Between the major proteins (SERCA and NCX) that determine Ca extrusion, NCX has been recognized to contribute more during CaT late phase²⁵ due to membrane potential being lower than the equilibrium potential of NCX ($E_{Na/Ca}$).²⁶ When NCX is over-expressed, β_2 -stimulation up-regulates its activity through β_2 -AR coupling to G_i protein.²⁷ However, this increase is not supported by our result (Figure 5G). Given that NCX is overexpressed in heart failure (Supplemental Figure 6), we postulate that reduced β_2 -regulation may be due to decreased β_2 -AR- G_i coupling.

Transmural remodeling of β_2 -AR regulation and DAD

In contrast to APD regulation, β_2 -AR system did not enhance Ca handling in failing hearts nor increased transmural CaD heterogeneity in either group. This disparity resulted in increased CaD-APD difference, which exacerbated DAD initiation. Here, we show DAD triggered PVCs associated with Ca release preceding AP upstroke in failing human LV myocardium. Interestingly, such calcium-mediated PVCs were only observed during β_2 -stimulation. PVCs were localized at the epicardium and midmyocardium, where (CaD-APD) is prolonged. In heart failure, given NCX activity has not been further augmented by β_2 -stimulation due to receptor uncoupling from G_i , the substrate (CaD-APD) could play a crucial role in DAD initiation (Figure 8A-B). In contrast, β_1 -stimulation did not provoke DADs in failing hearts.

Purkinje fibers are responsible for the majority of automaticity during β -stimulation in heart failure

Low frequency PVCs in normal hearts are considered benign, but frequent PVCs in failing hearts are associated with LV dysfunction.²⁸ In our study, the majority of PVCs originated from the Cx40 positive subendocardium. This suggests that PVCs evoked by β_1 - and β_2 -AR

stimulation arise in the Purkinje fibers in heart failure. Furthermore, it indicates that both subtypes may preserve the functional state of Purkinje fibers, different from that of working myocytes. Additionally, β_2 -stimulation also evokes DAD-induced triggered activity in the subepicardium and midmyocardium. Therefore, only β_2 -stimulation, and not β_1 -stimulation, leads to increased arrhythmia vulnerability by facilitating arrhythmogenic triggers and substrates. This specificity suggests that non-selective or β_2 -selective blockers may be more effective in treating and preventing arrhythmogenesis in heart failure than β_1 -selective blockers.

Limitations

A possible limitation of our study is our use of specific β_1 - and β_2 -agonists instead of a commonly used non-specific agonist combined with isoform-specific antagonists. We selected isoform-specific agonists Procaterol ($1\mu\text{mol/L}$)¹² and Xamoterol ($1\mu\text{mol/L}$)¹³ because of their established specificity in the settings of heart failure. Furthermore, it was not possible to do a dose response investigation due to the limited number of human hearts and limited time available for experiments. We decided not to use an alternative approach based on Isoproterenol combined with β_1 - and β_2 -blockers because we wanted to investigate the two types of receptors in the same heart, with access to a limited number of hearts. We acknowledge that lack of dose response data limits our ability to compare the relative contributions of β_1 and β_2 to EC coupling. Therefore, our comparisons were only between donor and failing hearts. Furthermore, we confirmed the selectivity of β_1 agonist Xamoterol, which had dramatic stimulation effects in donor but not in failing hearts, consistent with previous findings of desensitization of β_1 -AR in heart failure.⁹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Effects of β -stimulation on CV and anisotropy. A) Activation map illustrates CV measurements as a function of direction. B) Averaged wavefronts at each condition from donor and failing hearts are shown. CV_L values for C) donor and D) failing hearts are shown compared with baseline values prior to stimulation. Graphs of CV_T for E) donor and F) failing hearts with both stimulation conditions compared with baseline values. G) Donor and H) failing heart conduction anisotropy values. β_1 -stimulation increased anisotropy in donor hearts but decreased it in failing hearts, while β_2 -stimulation increased anisotropy in failing hearts. Box outlines show the 25th and 75th percentiles, and the middle lines indicate median values. Whiskers show the 5th and 95th percentiles.

Figure 2.

APD restitution curves and transmural gradients. A) Representative optical APs and APD maps recorded at 1Hz pacing are shown for different conditions. B) Restitution curves of average APD over the entire field of view are shown. C) APD restitution curves from all layers are shown. D) Transmural gradients of repolarization ($APD_{Endo} - APD_{Epi}$) at 1Hz pacing are shown for β_2 (upper panel) and β_1 -stimulation (lower panel).

Figure 3.

Effects of β-adrenergic stimulation on repolarization and remodeling of β₂-AR protein expression. A) AP signals showing that APD from β-stimulation are quantified as APD_{baseline}-APD_{β-stimulation}. B) Representative APD maps of donor and failing hearts are shown. C) and D) Statistical analysis of APD shows desensitization of β₁-AR and sensitization of β₂-AR at the endo and midmyocardium. Negative values indicate the reduction. E) Western blot shows a reversal of epi/endo gradient of β₂-AR protein expression in HF. F) Phosphorylation of β₂-AR is reduced in HF.

Figure 4.

Effects of β -stimulation on CaT and CaD-APD. A) Representative CaT signals. B) Representative CaD₈₀ maps. C) and D) Statistical analysis of Δ CaD reveals partial desensitization of β_1 -AR but not significant change of β_2 -AR regulation in HF. E) Calculations of Δ (CaD-APD) are shown. F) and G) Statistical analyses of Δ (CaD-APD) are shown.

Figure 5.

Morphological remodeling of CaT. A) Baseline, B) β_2 , and C) β_1 CaD₃₀/CaD₈₀ ratios displayed for each transmural region, with D) representative CaD₃₀/CaD₈₀ ratio maps. Transmurally averaged CaD₃₀/CaD₈₀ ratios for E) β_2 and F) β_1 experiments. F) T₅₀ and G) Ca²⁺ decay constants at baseline and after β_2 -stimulation are shown.

Figure 6.

Multiple mechanisms of automaticity and PVC in HF. A) Multiple types of PVCs were observed after β_2 -stimulation. AP amplitude maps were constructed at T1 and T2 time points to determine the location of the origination of both types of PVCs B) Ca-driven PVC was confirmed by AP-CaT delay map and found to share the same location with PVC#1. Activation times from two locations were labeled: PVC origination (Point #1 and 3 on AP and CaT activation map, respectively) and an adjacent location (#2 and 4). Activation time of 3 (CaT, 22ms) is earlier than 1 (AP, 26ms), and this difference is reversed shortly after AP propagated: activation time of 2 (AP, 28ms) is before 4 (CaT, 30ms). C) Enhanced automaticity shared the same location as PVC#2. D) Immunofluorescence staining of Cx40 and DAPI. Cx40 staining was observed in coronary vascular endothelium (Top-left) and endocardium (Middle-right). The staining is negative in epicardium (Bottom-left). The region of enhanced automaticity (small yellow box) expressed Cx40 (Top-right). Intramural tract branch of Purkinje fibers penetrating into myocardium was also observed (Bottom-right).

Figure 7. Ectopic activity characterized by location and frequency. A) Representative rapid pacing induced ectopic activity (VT) with AP, CaT, and MAP are shown. B) Location distribution of ectopic activity. C) Statistics of induced VT and ectopy. D) and E) Expanded view of ectopy and VT occurrence are shown.

Figure 8.

Illustrated schematic summaries of β -AR signaling remodeling and electrophysiological effects in heart failure. A) Schematic highlighting the switch of β_2 -AR coupling from G_i to G_s proteins, which alters downstream regulation from a negative to a positive feedback control system. B) Summary graphic of LV electrophysiologic responses to β_2 - and β_1 -stimulation in HF.