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The role of β-adrenergic system remodeling in human heart failure: A mechanistic investigation

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ABSTRACT

β-adrenergic receptor antagonists (β-blockers) are extensively used to improve cardiac performance in heart failure (HF), but the electrical improvements with these clinical treatments are not fully understood. The aim of this study was to analyze the electrophysiological effects of β-adrenergic system remodeling in heart failure with reduced ejection fraction and the underlying mechanisms. We used a combined mathematical model that integrated β-adrenergic signaling with electrophysiology and calcium cycling in human ventricular myocytes. HF remodeling, both in the electrophysiological and signaling systems, was introduced to quantitatively analyze changes in electrophysiological properties due to the stimulation of β-adrenergic receptors in failing myocytes. We found that the inotropic effect of β-adrenergic stimulation was reduced in HF due to the altered Ca^{2+} dynamics resulting from the combination of structural, electrophysiological and signaling remodeling. Isolated cells showed proarrhythmic risk after sympathetic stimulation because early afterdepolarizations appeared, and the vulnerability was greater in failing myocytes. When analyzing coupled cells, β-adrenergic stimulation reduced transmural repolarization gradients between endocardium and epicardium in normal tissue, but was less effective at reducing these gradients after HF remodeling. The comparison of the selective activation of β-adrenergic isoforms revealed that the response to β_2-adrenergic receptors stimulation was blunted in HF while β_1-adrenergic receptors downstream effectors regulated most of the changes observed after sympathetic stimulation. In conclusion, this study was able to reproduce an altered β-adrenergic activity on failing myocytes and to explain the mechanisms involved. The derived predictions could help in the treatment of HF and guide in the design of future experiments.

1. Introduction

Heart failure (HF) is a major health problem. Patients with reduced ejection fraction present weak cardiac performance and have a high risk of sudden cardiac death due to ventricular arrhythmias. Many alterations to the myocardium that occur in HF can promote the electrical instabilities observed. These include electrophysiological and structural remodeling of cardiac tissue, such as changes in the expression and function of membrane ion channels and Ca^{2+}-handling proteins. These changes can alter action potentials and Ca^{2+} dynamics and lead to the electrical and contractile dysfunction that characterizes failing myocytes [1,2]. Remodeling of signaling cascades, such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and β-adrenergic receptor (β-AR) pathways, also plays a crucial role [3,4].

Several neurohormonal mechanisms are activated during HF to maintain cardiac output, including the sympathetic nervous system, and a continuous release of catecholamines provokes a sustained activation of cardiac β-ARs. Catecholamine binding to membrane β-AR, a type of G-protein-coupled receptor (GPCR), initiates the adenylyl cyclase (AC)/ cyclic AMP (cAMP)/ protein kinase A (PKA) signaling cascade. PKA phosphorylates several electrophysiological proteins and modulates the electrical activity of the heart [5]. In the normal myocardium, this sympathetic stimulation increases heart rate and the force of contraction to enhance cardiac performance in demanding situations. In the failing heart, however, prolonged stimulation becomes detrimental and ultimately contributes to the pathogenesis of HF [6–8].

Abbreviations: AP, action potential; β-AR, β-adrenergic receptor; CaT, Ca^{2+} transient; cav, caveolar signaling domain; cyt, cytosolic signaling domain; ecav, extracaveolar signaling domain; HF, Heart failure; iso, isoproterenol; SR, sarcoplasmic reticulum; TDR, transmural dispersion of repolarization.

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Malignant arrhythmias are the major cause of death among patients with HF, and the use of β-blocker agents has demonstrated efficacy in reducing mortality rate and improving clinical outcomes [9,10]. Although changes in β-ARs and other proteins of the signaling cascade have been reported in failing myocytes, most clinical trials quantify the survival benefits of β-blocker therapy without going into depth regarding the mechanisms by which these drugs prevent arrhythmogenesis [11]. The existence of β-AR isoforms (β_1 and β_2-ARs are the major subtypes) with different pathways (e.g. only β_2-AR subtypes activate inhibitory G-proteins, and β_1 receptors are the only direct effectors on cytosolic protein phosphorylation) has raised questions related to variable effectiveness of β-blockers depending on drug affinity to the receptors. For instance, non-selective pharmacological agents or even partial agonists appear to have greater benefits than selective β_1 antagonists [12–14].

Investigations about cAMP signaling have given rise to detailed mathematical models of the adrenergic pathways, including signaling compartmentation and the interaction with electrophysiological proteins [15–17]. Simulations including these models can improve mechanistic insight into HF pathophysiology, but investigations performed to date have generally focused on effects of electrical remodeling rather than on HF-induced alterations in the β-AR system. The aim of this study was to apply β-adrenergic signaling changes according to experimental observations of protein function and expression in failing myocytes to reproduce the electrophysiological response to β-AR stimulation in HF. This approach allows the investigation of the altered signaling mechanisms that trigger electrical instabilities and contractile dysfunction in patients with HF with reduced ejection fraction. We integrated existing cellular models of signaling and electrophysiology and introduced changes in protein expression and localization according to available data. These initial results will be useful to understand mechanisms and guide future experimental studies aimed at improving clinical HF treatments.

2. Methods

2.1. Baseline cellular models

The mathematical model representing a human ventricular myocyte included the Heijman et al. β-adrenergic signaling pathway [17] integrated into the ORd action potential model [18], which also considers the CaMKII signaling cascade. The complete formulation was initially described in [19] to represent a normal epicardial cell. We used an improved version of the model [20] which maintained the main characteristics, such as cAMP signal compartmentation and the PKA-mediated phosphorylation of eight electrophysiological proteins: L-type Ca^{2+} channel (I_{CaL}), slow delayed rectifier K^+ channel (I_{Ks}), phospholamban (PLB), troponin I (TnI), ryanodine receptor (RyR), fast Na^+ channel (I_{Na}), Na^+/K^+ pump (I_{NaK}), and background K^+ current (I_{Kb}).

To simulate HF with reduced ejection fraction phenotype (referred only as HF from now on), several changes were introduced in the model. The electrophysiological part was modified to represent the characteristic HF modifications at the cellular level, such as ion channel remodeling and the loss of T-tubular cellular domains. Heterogeneous HF remodeling was implemented as indicated in Gomez et al. [2]: upregulation of the late Na^+ current, the Na^+/Ca^{2+} exchanger, the sarcoplasmic reticulum (SR) Ca^{2+} leak, and the CaMKII, and downregulation of the SR Ca^{2+} uptake (SERCA), the transient outward and inward rectifier K^+ currents, the Na^+/K^+ ATPase current, and the Ca^{2+} release sensitivity via RyRs. Regarding detubulation, several changes were introduced following Sanchez-Alonso et al. [21] methodology: (i) L-type calcium channels (LTCC), which are predominant in the subspace (submembrane space near T-tubules), were redistributed into the crest, (ii) half of the dyadic Na^+/Ca^{2+} exchangers (NCX) were relocated to the surface membrane, and (iii) Ca^{2+} release from the SR was reduced to reproduce orphaned RyRs.

2.2. Signaling remodeling in heart failure

The formulation of the β-AR signaling model was also modified to reproduce the alterations in HF. An extensive review of experimental observations is summarized in Table S2. Despite the general agreement on the increased or decreased expression and function of proteins, we found a wide range of variation and considered it instead of taking a fixed value. A better adjustment was performed in several steps, described below.

Fig. 1 summarizes the remodeling in β-adrenergic signaling: β_1-ARs are downregulated, which reduces β-AR responsiveness; β_2-ARs migrate from caveolar (cav) to extracaveolar (ecav) domain due to detubulation without causing receptor loss; there is G protein-coupled receptor kinase (GRK) upregulation, which phosphorylates β-ARs and this desensitization implies β-AR loss of function; phosphodiesterase type 4 (PDE4) is downregulated, inhibiting less cAMP and promoting PKA-phosphorylation; the downregulation of the cytosolic (cyt) inhibitor1 increases phosphatase type 1 (PP1) activity, which further dephosphorylates PLB, decreasing SERCA function. T-tubule degradation causing β_2-ARs signaling disruption affects I_{CaL} phosphorylation leading to new ecav signaling pathways for subsarcolemmal LTCC (I_{CaLe}).

Additional modifications were performed to differentiate between myocardium layers. ORd model already considers transmural heterogeneity. We applied different β_2-AR protein expression along the transmural wall cells, as quantified by Lang et al. [22], to simulate endocardial cells in addition to epicardial cells (Table S3). There were also transmural differences between normal ($\beta_2\text{-AR}_{epi} < \beta_2\text{-AR}_{endo}$) and failing myocytes ($\beta_2\text{-AR}_{epi} > \beta_2\text{-AR}_{endo}$). Further details of the model can be found in the supplemental material.

The modifications implemented in the signaling model were first of all calibrated with experiments [22]. From the literature (Table S2), we had a qualitative knowledge about the 5 important HF remodeling changes in the β-AR system. To quantify the molecular changes that led to HF phenotype, a population of human epicardial failing cells was constructed with uniform variability in the signaling parameters. We selected the cells that underwent the same electrophysiological variations as in experiments [22] according to the following criteria: action potential duration (APD) and CaT duration (CaTD) in epicardial cells upon β-AR stimulation with isoproterenol were reduced compared to the non-stimulated cells in a specific range ($-25\% < \Delta APD_{80} < -13.2\%$ and $\Delta CaTD_{80} < 0\%$).

2.3. Single cell simulations

2.3.1. β-adrenergic stimulation protocol

β-AR stimulation was simulated with saturated dose of agonists, i.e. the concentration at which the activation of the receptors is maximal so that results were dose independent (1 μM). Besides, model equations were run until achieve steady-state conditions to ensure the stability of signaling variables modulating the electrophysiology. Heijman et al. β-adrenergic model [17] was defined for the non-selective agonist isoproterenol, although in the formulation the activation of β_1 and β_2-ARs isoforms was differentiated. We performed selective simulations in which only one receptor subtype was stimulated as if the other was blocked. The use of isoproterenol and specific antagonists is the common approach in most of experiments, facilitating the comparison.

2.3.2. Population of models

Biological variability is known to be a feature that can vary the response of different individuals to the same perturbation such as a drug or pathology. For this reason, we considered in this study populations of cells instead of only the baseline models. We applied parameter variability to electrophysiological and β-adrenergic remodeling variables with scaling factors following a log-normal distribution (59%–169%). A total of 300 different cells were initially generated and calibrated to match physiological biomarkers ranges [23]. Medians and interquartile

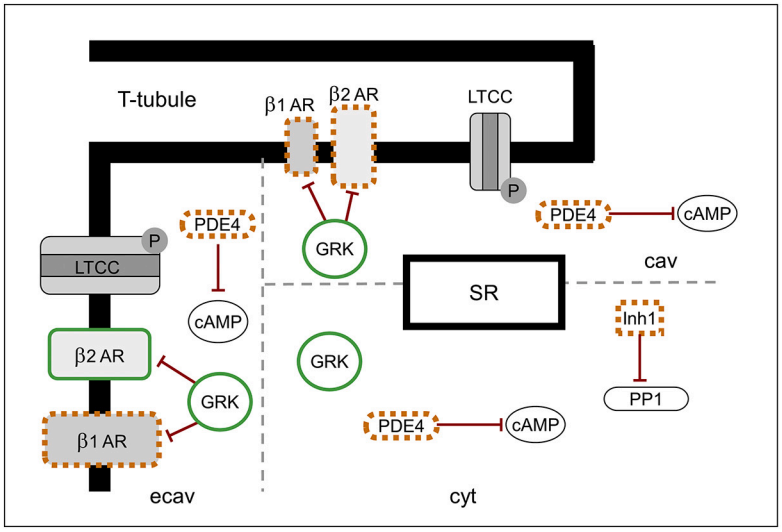

Fig. 1. β-adrenergic signaling remodeling in heart failure (HF). Downregulated proteins: isoform β_1 adrenoreceptor (β_1-AR), phosphodiesterase 4 (PDE4) and inhibitor 1 (Inh1); upregulated proteins: G protein-coupled receptor kinase (GRK). Other changes: redistribution of β_2 adrenoreceptor (β_2-AR) activity from caveolar (cav) to extracaveolar (ecav), L-type Ca^{2+} channels (LTCC) that migrate to the crest are phosphorylated by ecav signaling pathways. Molecules of cytosolic (cyt) compartment are also affected.

ranges were measured and EADs cases were excluded from calculations because of the repolarization abnormalities.

The statistical analysis was performed using the Wilcoxon rank sum test in MATLAB. Parameters differences were statistically significant for p-values <0.01.

2.3.3. Phosphorylation of electrophysiological substrates

The phosphorylation of each substrate was represented by a fraction ranging from 0 (basal PKA-phosphorylation) to 1 (maximum). To study the individual electrophysiological effects of phosphorylated proteins, we performed a univariate contribution analysis, in which one substrate was completely phosphorylated while the others remained at basal levels. APD and CaT peak sensitivities to each parameter were then quantified for both the N and HF baseline model. Phosphorylation fractions resulting from each baseline model with the different modes of β-AR stimulation were also analyzed to compare the contribution of phosphorylated proteins in each case.

2.3.4. Electrophysiological indicators

The last AP and CaT of 300 beats were examined, after assuring the stabilization of both the β-adrenergic system and the electrophysiological activity. Cellular biomarkers were APD, calculated at 80% and 90% of repolarization (APD_{80} and APD_{90}), CaT peak (CaT_{max}), CaT duration from maximum upstroke to 80% recovery ($CaTD_{80}$), and the interval between electrical and mechanical termination computed as the difference $CaTD_{80}$-APD_{80}. All simulations were run at 1 Hz and biomarkers were obtained for this frequency.

2.3.5. Transmural strand simulations

A one-dimensional cable composed of 166 myocytes was used to study the electrical activity and propagation in coupled cells [18] and was simulated in Elvira, a software to solve the monodomain equation by using the finite element technique of the operator splitting [24]. One half of the strand was composed of endocardial cells and the other half of epicardial cells. Midmyocardial cells were not used because of their controversial behavior [25,26]. Instead, we applied a gradient of 20 cells between endocardial and epicardial to smooth the transition. An additional HF remodeling change considered is the reduction of intercellular coupling modeled by a 50% decrease in the diffusion coefficient [2]. To avoid edge effects, only the 146 central nodes were considered for calculations. Besides cellular biomarkers, we measured repolarization time (RT) along the fiber as time to 90% of repolarization since the initiation of the stimulus for each cell. The difference between the maximum and minimum RT values indicated the transmural dispersion of repolarization (TDR).

3. Results

3.1. β-Adrenergic stimulation in heart failure

In failing myocytes, in addition to the electrophysiological remodeling, changes in β-adrenergic signaling proteins may contribute to the altered electrophysiology. Therefore, the initial goal was to obtain insight into this signaling remodeling by establishing specific changes that may best represent the HF phenotype. From previous experimental studies, we selected 5 model parameters commonly reported to be altered in HF and generated a population in which these were upregulated or downregulated within a defined variability range (Table S2). Fig. 2A, showing APs and CaTs from these HF populations, illustrates that β-adrenergic stimulation with isoproterenol modulated differently the electrophysiological phenotype depending on parameter combinations applied for signaling remodeling (blue traces). The same remodeling also affected, to a lower extent, APs and CaTs of cells with no β-stimulation (green traces). From these populations we selected cells that showed consistency with experiments [22] in the degree of APD and CaTD shortening following β-stimulation (Fig. 2B), as described in the Methods section. This calibration step was used to define the signaling HF remodeling.

During the calibration, 324 out of the initial 1000 models were within the accepted group. Ranges of the 5 signaling parameters in the accepted and rejected sets are shown in Fig. 2C. Since differences between groups were all statistically significant ($p < 0.01$), we chose the median values of the 5 parameters from the accepted models, shown in Table 1 as scale factors relative to normal myocytes, to define the basic β-adrenergic remodeling in HF. Specifically, this analysis quantitatively predicts downregulation of β_1-ARs, PDE4 and Inh1, upregulation of GRK upregulation, and a decrease in β_2-ARs in the cav compartment due to their migration to the ecav domain. These changes, together with the electrophysiological and structural remodeling in myocytes, constituted our baseline HF model.

3.2. Comparison of β-adrenergic stimulation in normal and failing myocytes

In Fig. 3, the effects of β-adrenergic stimulation with isoproterenol are compared in normal and failing myocytes. Fig. 3A represents the AP and CaT traces for the baseline models under the four different conditions: N or HF, and with or without isoproterenol. When comparing the

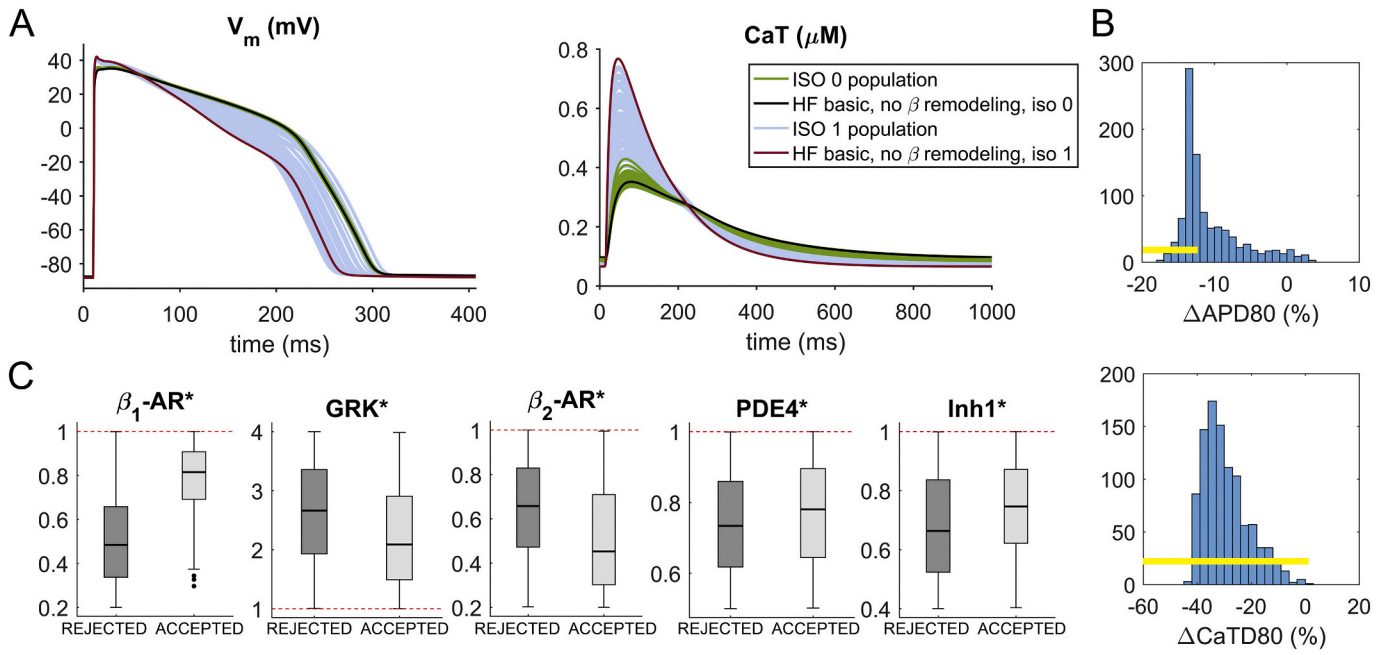

Fig. 2. Experimental calibration of a β-population of failing (HF) myocytes stimulated with isoproterenol (iso). A) Action potential (AP) and Ca^{2+} transient (CaT) traces of some representative cells of the population with fixed electrophysiological remodeling and variable signaling. The basic HF models (black and red, for iso0 and iso1 respectively) represent the APs and CaT of myocytes without modifications in the β-adrenergic system. B) Biomarker histograms of the population show ΔAPD_{80} and $\Delta CaTD_{80}$ ranges accepted. C) Signaling parameter distributions divided in accepted and rejected models ($*p < 0.01$). Results from epicardial cells stimulated at 1 Hz. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
β-adrenergic system remodeling in heart failure.

Molecule	Median (scale factors)
β_1-AR	0.82
GRK	2.09
β_2-AR	0.45
PDE4	0.78
Inhibitor1	0.75

electrophysiology between normal and failing cells, the characteristic long AP and reduced systolic Ca^{2+} in HF were also observed following sympathetic stimulation. In HF, β-adrenergic stimulation produced slightly less APD_{90} shortening (reduction of 12% versus 15% in normal myocytes) and a smaller relative increase of CaT amplitude (increase of 89% versus 108%).

Population simulations were then conducted to evaluate variability in the response to β-stimulation in N and HF myocytes. Inter-individual variability in signaling and electrophysiological parameters introduced dispersion to each of the studied biomarkers (Fig. S1) and allowed us to quantify the vulnerability to repolarization abnormalities in the different cases (Fig. 3B). Whereas cells without β-adrenergic stimulation were not prone to develop EADs, isoproterenol caused repolarization abnormalities.

Transmural dispersion of repolarization is strongly related to arrhythmogenesis, and we therefore analyzed whether there were differential effects of β-stimulation in epicardial versus endocardial cells. Our simulations showed that endocardial cells were more vulnerable to EADs under β-stimulation than epicardial cells, and failing conditions increased the probability of EADs development in both types of cells. The repolarization difference between endocardial and epicardial cells, shown in Fig. 3C and measured as APD_{90} dispersion ($\Delta APD_{90endo\text{-}epi}$), decreased with isoproterenol for N and HF myocytes similarly. In the case of HF, β-adrenergic stimulation counteracted the increase caused by failing conditions. Regarding CaT, the increase of Ca^{2+} peak caused by sympathetic stimulation depended on the maximum Ca^{2+} in control conditions. In HF, the depressed CaT was slightly enhanced and the increase was smaller in endocardial cells, which have less systolic Ca^{2+} than epicardial cells.

The delay between Ca^{2+} recovery and APD repolarization, quantified as ($CaTD_{80}$-APD_{80}), has been proposed as a biomarker of arrhythmia vulnerability [22,23], as delayed afterdepolarizations (DADs) can be triggered by the elevated intracellular [Ca^{2+}] after the membrane has already repolarized. Fig. 3C shows that β-adrenergic activity shortened CaTD-APD interval, thereby presumably reducing the predisposition to DADs. Compared to normal conditions in which major reductions of CaTD-APD period were obtained after sympathetic stimulation, the reduction effect was smaller in HF, especially in endocardial cells. Electrophysiological HF remodeling was the cause of the elevated CaTD-APD, and this combined to the altered β-adrenergic regulation led to a smaller reduction compared with normal cells, and potentially increased susceptibility to DADs.

These results highlight the differential effects of β-AR stimulation on N and HF myocytes. Although isoproterenol is protective in N and HF conditions by reducing APD dispersion, the impact on decreasing the probability of DADs is smaller in HF and the arrhythmogenic risk of HF through EADs development increases with β-AR stimulation. Isoproterenol also improves CaT in HF but the increase is far from the effect in N cells.

3.3. Selective β-adrenergic stimulation

The understanding of the relative role of β-AR isoforms on electrophysiology is crucial to select among a variety of pharmacological agents. In HF, β_1 isoform is reduced while β_2 subtype changes its location in the membrane compartments without altering the total number of receptors. The differences in the remodeling of β_1 and β_2 pathways help explain the differential roles of both isoforms in the altered electrophysiological behavior of failing cells. The individual influence of the two dominant subtypes on cardiac electrophysiology were examined to evaluate potential differences between them and a non-selective

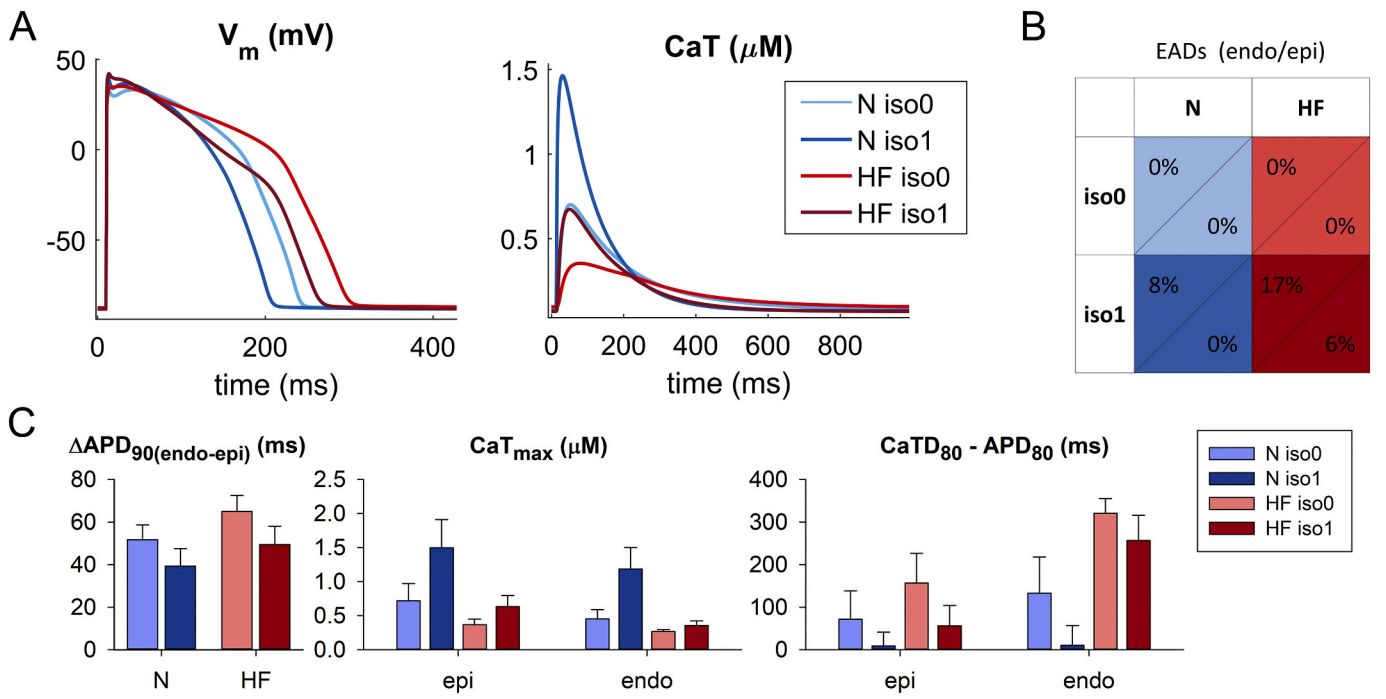

Fig. 3. β-adrenergic modulation of action potential (AP) and Ca^{2+} transient (CaT) in normal (N) and failing (HF) myocytes. A) Comparison of AP and CaT traces of the baseline epicardial models. B) Quantification of early afterdepolarizations (EADs) cases in N and HF populations of endocardial (endo) and epicardial (epi) cells. C) Comparison of transmural APD dispersion ($APD_{90endo-epi}$), CaT peak (CaT_{max}) and $CaTD_{80}$-APD_{80} interval in the population (median and interquartile range). Results from cells stimulated at 1 Hz.

stimulation. Fig. 4A compares AP and CaT traces in failing myocytes under different signaling conditions. The electrophysiological effect of the activation of both receptor subtypes simultaneously ($\beta_1 + \beta_2$, equivalent to isoproterenol) was very similar to selective β_1-AR stimulation, and differed from selective β_2-AR stimulation, which caused only minimal changes to membrane potential or intracellular [Ca^{2+}].

In Fig. 4C, the population response to β-adrenergic stimulation was quantified as the median of the difference of biomarkers between β-ARs stimulation and control (without β-AR stimulation) for every cell. Negative values indicate a decrease and positive values an increase in

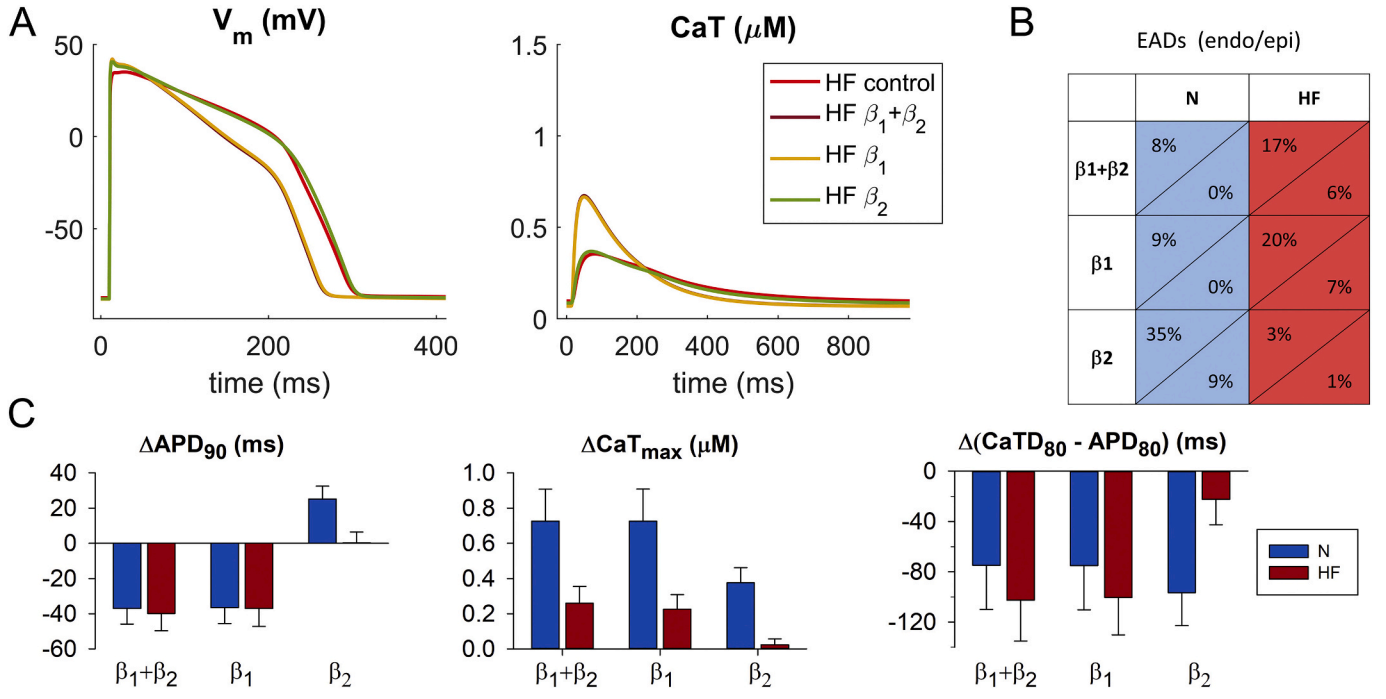

Fig. 4. Selective β-adrenergic modulation of action potential (AP) and Ca^{2+} transient (CaT) in heart failure (HF). A) Comparison of AP and CaT traces of the HF baseline model. B) Quantification of early afterdepolarizations (EADs) cases in N and HF populations of endocardial (endo) and epicardial (epi) cells. C) Comparison of β-isoform effect on APD90, CaT peak (CaT_{max}) and $CaTD_{80}$-APD_{80} interval in the population (median and interquartile range). Results from epicardial cells stimulated at 1 Hz.

the corresponding biomarker. APD reduction, CaT increase and ($CaTD_{80}$-APD_{80}) decrease were observed with specific β_1-AR stimulation and this modulation was very similar to the one exerted by isoproterenol. This occurred both in HF and N conditions. However, β_2-AR stimulation provoked changes of less or opposite magnitude (shortening instead of prolongation in some cell models). For instance, while β_2 modulation of CaT was half of β_1 effect in N myocytes, it was blunted in the setting of HF. This minimal impact was also observed in the other biomarkers. Surprisingly, while isoproterenol shortened APD, β_2 agonists prolonged it in normal myocytes. This prolongation was also related to the increased number of EADs with β_2-AR stimulation. However, our results suggest that β_2 becomes less proarrhythmic in HF in terms of EAD generation, whereas β_1 increases the probability of EADs. Therefore, the arrhythmogenic effects (EADs) of β-AR stimulation in HF are due to the β_1-AR subtype, but the activation of β_1-AR pathways is also the main way to increase Ca^{2+} levels and reduce the CaTD-APD interval via the β-adrenergic system.

The analysis of EADs to elucidate which β-adrenergic parameters contributed to these repolarization abnormalities were not conclusive and only electrophysiological properties could be related to this phenotype as in previous studies (see supplemental Fig. S3).

3.4. Mechanisms of β-adrenergic signaling

The modulation of the electrophysiological activity of cardiomyocytes by β-AR signaling cascades is mediated by PKA phosphorylation of membrane ion channels and Ca^{2+}-handling proteins. A contribution analysis of the eight targets subject to phosphorylation was performed to quantify their individual impact on APD_{90} and CaT_{max}. Fig. 5A shows, as percentages, the univariate contribution of each PKA-phosphorylated target to APD_{90} and CaT_{max}. These were obtained through simulations in which the phosphorylation fractions of individual targets were set to 1 while the others were fixed at 0. I_{Ks} turned out to be the main modulator of APD shortening, followed by I_{CaL} and I_{NaK} with prolongation effects. I_{CaL} and PLB were the key factors responsible for CaT_{max} increase under β-AR stimulation. Different values between N and HF highlight that electrophysiological remodeling alters the response to protein phosphorylation. The most remarkable observation is that I_{CaL} impact decreased considerably in HF. This is because I_{CaL} refers only to channels PKA-phosphorylated in the cav domain, and it was compensated by the contribution of I_{CaL} phosphorylation in the ecav domain (I_{CaLe}), as L-type Ca^{2+} channels distribute in this domain in HF due to loss of T-tubules.

Apart from sensitivities, the activating effect of PKA phosphorylation on electrophysiological substrates also depends on the degree of phosphorylation. Fig. 5B represents the PKA-phosphorylation level of each electrophysiological substrate in N (left) and in HF (right) conditions, differentiating between non-selective stimulation ($\beta_1+\beta_2$) and selective β-AR isoform stimulation (blue, orange and green bars respectively). A fraction equal to 1 represents a maximal change in the target protein phosphorylation, while 0 represents the baseline phosphorylated state. Differences between N and HF are due to β-adrenergic protein remodeling in failing myocytes (β_1-AR downregulation, GRK upregulation, etc) which alter compartmentalization and PKA-phosphorylation activity. Maximal phosphorylation fractions were computed under non-selective and β_1-selective stimulation in normal cells. In HF, a slight decrease in the eight original targets was observed, while I_{CaLe}, only present in failing cells, increased. In N conditions, saturated selective β_2-AR stimulation only led to maximal phosphorylation of caveolar substrates, highlighting the local PKA activation produced by β_2-ARs. Changes from N to HF are more remarkable under β_2-selective stimulation because extracaveolar phosphorylation of substrates increases and the activation tends to be more global.

This analysis highlights on the one hand, that HF electrophysiological remodeling alters phosphorylation impact on AP and CaT, and on the other hand that β-adrenergic signaling remodeling, as well as selective β-AR stimulation, changes substrate phosphorylation levels. Both systems contribute to the final electrophysiological phenotype of failing myocytes.

3.5. Variability of β-adrenergic signaling

The introduction of interindividual differences to consider all the potential electrophysiological phenotypes provided a wide range of biomarkers variability. Fig. 6 illustrates the histograms of biomarkers modulation as the difference between β-ARs stimulation and control.

In this way, all normal myocytes presented APD shortening after β_1-AR stimulation, but APD prolongation after β_2-AR activation. In the case of CaT_{max}, it was increased in all models, the only difference between β-ARs was the range of CaT_{max} variation. In HF, β-adrenergic remodeling

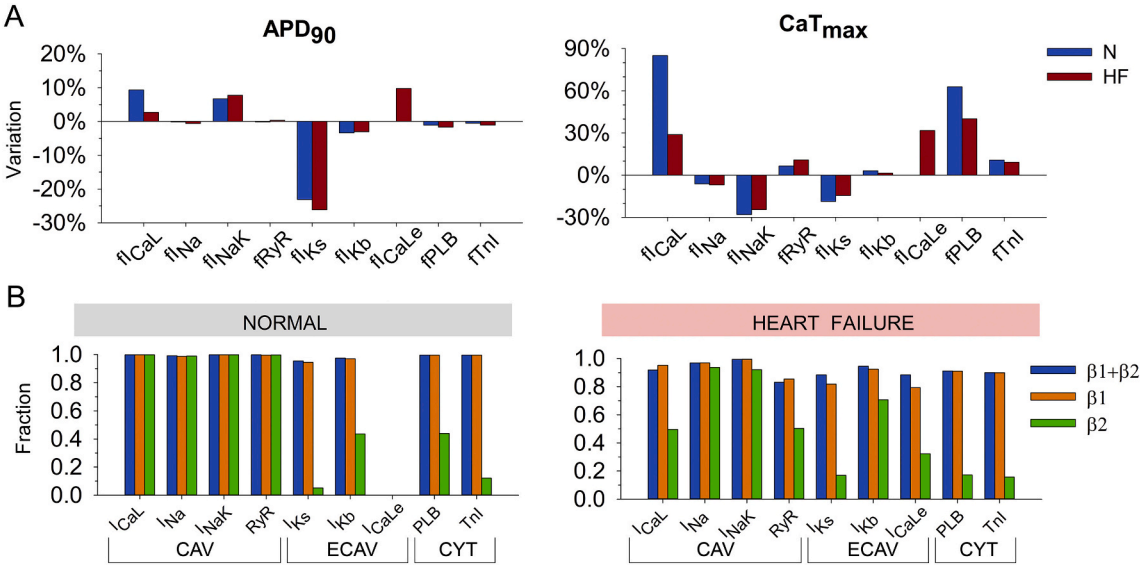

Fig. 5. Sensitivity analysis of βAR-mediated PKA-phosphorylation. A) APD and CaT_{max} sensitivities to individual substrate phosphorylation fraction ($f_{protein}$) in N and HF. B) Comparison of substrate PKA-phosphorylation under saturated selective and non-selective β-stimulation. Signaling compartmentation: caveolar (cav), extracaveolar (ecav) and cytosolic (cyt). Results from epicardial cells stimulated at 1 Hz.

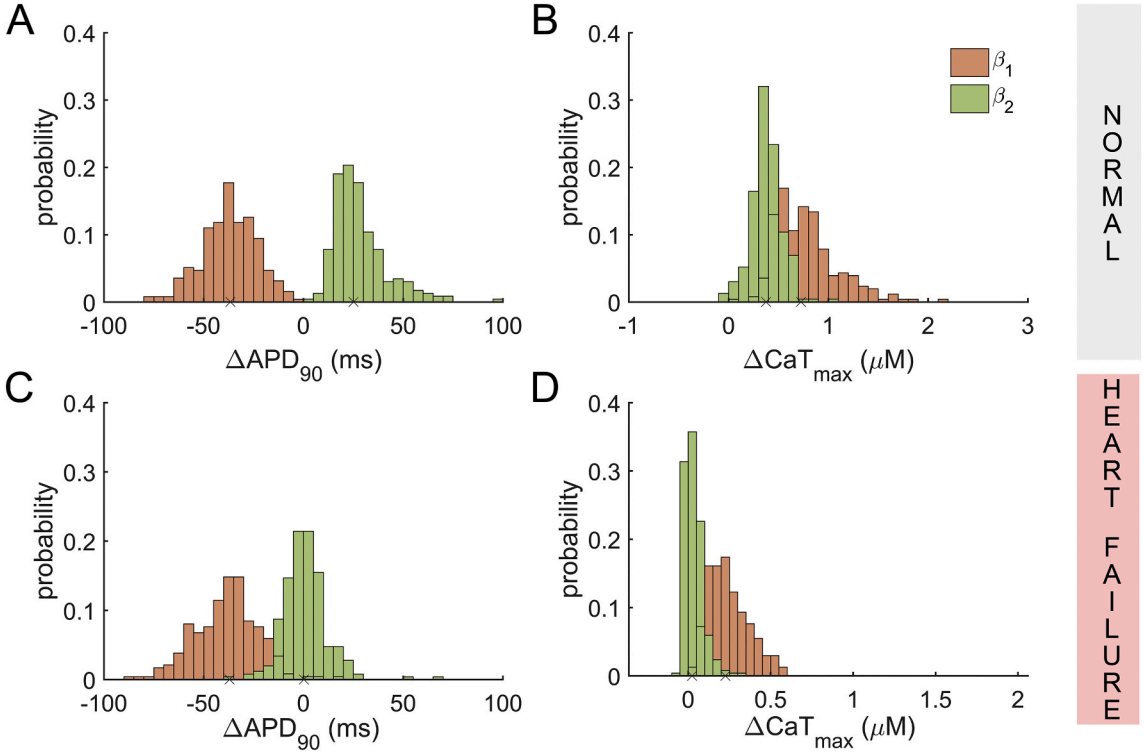

Fig. 6. Interindividual variability in selective βAR stimulation. A) APD and CaT modulation histograms in the normal (N) population. B) APD and CaT modulation histograms in the failing (HF) population. Results from epicardial cells stimulated at 1 Hz.

caused changes in β_2-AR modulation effect. Indeed, the increase or decrease of APD and CaT_{max} depended on the remodeling degree of the signaling parameters. To go in depth into this phenomenon, populations were separated in two groups according to the increase or decrease of biomarkers after selective β-AR stimulation (Fig. S2). Then, the contribution to this classification of the 5 signaling parameters remodeled in HF was analyzed. Fig. S2 panel A shows that β_2-AR and PDE4 controlled APD modulation; the strong downregulation of the former shortened it ($\Delta APD_{90} < 0$) while the strong downregulation of the latter prolonged the duration ($\Delta APD_{90} > 0$). The analysis of CaT_{max} in Fig. S2 panel B indicates that only strong PDE4 downregulation and weak downregulation of inhibitor1 contributed to CaT_{max} increase ($\Delta CaT_{max} > 0$).

Fig. S2 panel C helps explain the similarities between selective β_1 and non-selective stimulation (iso) by comparing biomarker differences. As expected, a strong downregulation of this receptor isoform, as can occur in HF, increased the differences between both stimulation protocols, highlighting the dominant role of β_1-AR in human cardiomyocytes.

Populations of models indicate that specific changes in the β-AR

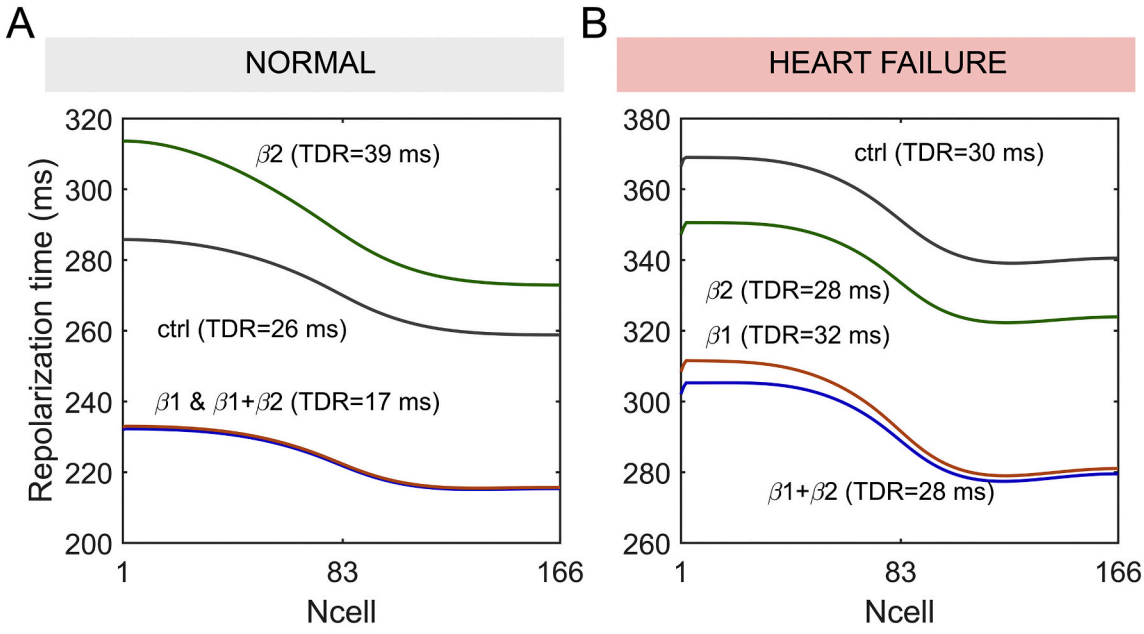

Fig. 7. Dispersion of repolarization in a transmural strand. Normal (N) vs heart failure (HF) for the different β-adrenoreceptor isoform stimulation conditions.

signaling pathway can lead to different β-AR stimulation responses, especially with β_2-AR.

3.6. Transmural effects of β-AR stimulation

Simulations in a multicellular one-dimensional cable including endocardial and epicardial cells complemented the unicellular simulations in the different types of cells by adding the effect of cellular coupling and transmural electrical propagation. As predicted with APD values in cellular simulations, repolarization time, measured as time to 90% of repolarization since the initiation of depolarization, decreased with β-AR stimulation for both N and HF conditions, except for β_2-AR in normal myocytes (Fig. 7). However, the effect on transmural dispersion of repolarization (TDR) differed between strands composed of normal or HF myocytes. While isoproterenol and selective β_1-AR stimulation reduced TDR in the normal strand, β-AR stimulation in HF had only minimal effects and would therefore not be beneficial. The control strand shows that the electrophysiological modifications and slow conduction velocity due to reduced intercellular coupling in HF predispose the myocardium to a greater risk of arrhythmia with larger repolarization times and TDR. The only exception was β_2-AR that became less arrhythmogenic in HF than in normal myocytes. All in all, what these results suggest is that the protective effects of β-AR stimulation (TDR reduction) observed in N myocytes disappear in HF.

4. Discussion

4.1. Main findings

The adverse changes in ventricular myocytes during the progression of HF lead to electrical instabilities and contractile dysfunction. According to this study, the role of the β-adrenergic system should be considered, since the electrophysiological modulation resulting from signaling protein remodeling might contribute to arrhythmogenesis. Additionally, the electrophysiological response of failing cells to sympathetic stimulation is different to the effects observed with β-agonists in normal myocytes, which highlights the need to test pharmacological agents under the setting of HF. The present computational study, based on the available data to date, predicts that in HF β-AR stimulation (i) induces a smaller positive inotropic response compared with that seen in normal myocytes, (ii) increases the vulnerability of myocytes to develop EADs, (iii) decreases the probability of DADs generation by reducing the CaTD-APD interval although, compared to N, HF is less protective, (iv) does not provide a beneficial reduction of TDR as observed in N conditions, and (v) is blunted when only β_2-AR isoforms are activated.

4.2. Compartmented PKA-phosphorylation activated by β-adrenergic stimulation modulates action potentials and Ca^{2+} transients

The β-AR-mediated modulation of AP and CaT determines the electrical activity and contractility of the heart. While the inotropic effect of β-AR agonists is widely known [27,28], the exact β-AR-modulation of APD is controversial: APD is prolonged [29,30] or reduced [22,31] depending on the study. It is to be noted that available data in the literature use different experimental protocols, drugs, and species, so that a wide variability in the results can be found. In the present study we obtained APD and CaTD shortening with isoproterenol as observed in ventricular wedge preparations of human hearts [22].

The analysis of PKA-phosphorylation degree explained that APD shortening resulted from the dominant effect of phosphorylated I_{Ks} while the increase of CaT_{max} (equivalent to CaTD shortening) was mainly due to the accumulated contribution of I_{CaL} and PLB phosphorylation. Selective β_2-AR isoform stimulation had a moderate inotropic effect due to the low PKA-phosphorylation of PLB compared to β_1-AR stimulation, and provoked APD prolongation due to the dominant effect of I_{CaL} phosphorylation versus minimal I_{Ks} phosphorylation. The location of β-ARs subtypes and the compartmentation of the signaling cascade were therefore critical factors regulating electrophysiological outcomes exerted by β-adrenergic stimulation. It is known that β_1-ARs are the prominent subtype in cardiomyocytes and generate global cAMP signals [27,32], which explains that the regulation mediated by isoproterenol and β_1-AR stimulation was identical in N conditions. It is to be noted that our simulation results showed APD prolongation with β_2-AR stimulation as opposed to the shortening observed by Lang et al. [22]. This discrepancy could be attributed to the fact that the drugs used (xamoterol and procaterol) as specific ligands for the activation of β-adrenergic isoforms in [22] could have partial specificity [12,33,34]. In fact, the effects of isoproterenol ($\beta_1 + \beta_2$) in our simulations were consistent with the experiments, and discrepancies appeared when comparing the effects of selective agonists. To see if the model can also reproduce the experimental results, a possible scenario of imperfect selective isoform activation was simulated and is included in the supplemental material. Results supported the hypothesis of the partial specificity offered by the experimental compounds and strengthened the contribution of computer models in assessing the effects of β-AR stimulation.

In failing myocytes, signaling remodeling alters PKA-phosphorylation and consequently has a different impact on AP and CaT, which is also determined by the electrophysiological remodeling. I_{Ks} phosphorylation seems to be critical to shorten the long APD in failing myocytes, which also explains the blunted effect of selective β_2-AR stimulation. β_2-AR stimulation did not cause maximal LTCC phosphorylation because cAMP signaling was less localized in the caveolar domain after HF remodeling, and part of I_{CaL} phosphorylation effect on APD and CaT was translated to the channels relocated in the sarcolemma after detubulation. In a recent study, Loucks et al. [35] studied in silico the degradation of T-tubular domains by redistributing LTCCs and cAMP signaling and showed that channels relocated to the sarcolemmal membrane in HF can produce sustained current that increases the risk of EADs. Regarding CaT, PLB phosphorylation acts as a compensatory mechanism to SERCA downregulation by enhancing SR Ca^{2+} uptake [36,37], but the increase effect on intracellular Ca^{2+} appears to be reduced in HF.

4.3. Adverse β-adrenergic remodeling effects in HF. Desensitization and detubulation

While sympathetic activity is initially a mechanism to increase contractility and maintain cardiac output in HF, the chronic stimulation becomes detrimental and initiates a protein remodeling process that disrupts the β-adrenergic signaling cascade [38]. Desensitization, controlled by PKA and GRK, is initially an adaptive mechanism [39] which in HF leads to increased phosphorylation and degradation of β-ARs [40]. When we incorporated β_1-AR downregulation and GRK upregulation in the HF model, decreased cellular cAMP levels provoked a reduction in PKA-phosphorylation of substrates with β-agonists in HF compared to N and explained the lower inotropy observed [27,41]. A β_1-AR downregulation higher than 18% (value applied in the baseline model of HF) would be realistic in an aggravated HF condition and could decrease PKA-phosphorylation to a larger extent, increasing differences in the electrophysiological modulation between non-selective and selective β_1-AR stimulation. The controversial modifications in the inhibitory G-protein (G_i) coupled to β_2-AR [22,42,43] during HF were not introduced. However, upregulated GRK, which enhanced protein function, contributed indirectly to reduce G_i activation. Both PKA and GRK compete in the phosphorylation of β-AR. As β_2-AR coupling to G_i depends on GPCR PKA-phosphorylation, a higher GRK-mediated phosphorylation reduced PKA-phosphorylation of receptors and β_2-ARs coupling to G-protein (G_s) dominated over G_i. These results agree with the reduction in PKA-phosphorylation of β_2-AR observed in failing myocytes and the consequent switch from G_i to G_s activation [22]. Nevertheless, the most important remodeling factor altering β_2-AR

signaling is detubulation.

Structural and functional remodeling of cellular microdomains leads to changes in cAMP compartmentation. β_2-AR signaling, which is locally confined in T-tubules and regulates LTCC in healthy myocytes, undergoes substantial changes in HF, presumably due to the loss of T-tubular microdomains [44,45], which can explain the diminished electrophysiological effects in HF. Other studies highlight though that due to the conservation of the total number of β_2-AR in HF, these receptors remain functional and cause positive inotropy and changes in Ca^{2+} [27,46,47]. When we introduced all the changes observed in cAMP signaling compartmentation [48–50], β_2-AR signaling became more global and the modulation of APD and CaT decreased. The tight regulation of I_{CaL} by β_2-AR in N conditions is weakened in failing myocytes because of the reorganization of channels and receptors, contributing to the impairment of Ca^{2+} dynamics. Besides detubulation, PDE downregulation also causes changes in the compartmented cAMP and PKA signals that affect AP and CaT [45,51]. Cytosolic proteins, such as PLB, were not further phosphorylated in HF following β_2-AR stimulation as DeSantiago et al. observed [46], in part because the enhanced activity of PP1 found in failing myocytes due to inhibitor1 downregulation restricts PKA activity [52,53], and because β_2-ARs still have limited control of cytosolic cAMP signals in the model despite applying caveolar signaling disruption [54,55].

4.4. Arrhythmogenesis in failing myocytes

Enhanced sympathetic activity has been related to the generation of arrhythmias, especially in HF, where the development of EADs and DADs has been observed [4,22,29,35,46]. Our simulations confirmed that EAD development increased after β-AR stimulation in HF. With respect to DAD generation, although our simulations did not show DADs, the wider CaTD-APD period in HF compared to control would suggest a higher likelihood of DAD development in HF. Previous studies have suggested that Ca^{2+} overload plays a role in afterdepolarization formation and that β_2-AR stimulation might be the most arrhythmogenic factor in HF [4,22,35,46]. Triggered activity caused by DADs were observed only during β_2-AR stimulation in human wedge preparations of failing hearts, which were attributed to transmural differences in CaT and APD generated after HF remodeling [22]. By contrast, in our simulations, both the inotropic effects of β_2-AR stimulation and the apparent arrhythmia susceptibility were reduced in HF compared with normal cells and tissues. These discrepancies could be due to the partial specificity of β-agonists used in experiments versus the theoretical approach in which only one β-AR subtype is activated while the other is completely blocked. In N conditions, EADs resulting from sympathetic stimulation are driven by APD prolongation due to I_{CaL} enhancement resulting from PKA-phosphorylation, but none of the β-AR system related factors was significant. This finding was also observed in the computational study of Loucks et al. [35], with the mechanistic difference that EADs were induced in failing myocytes after T-tubular disruption and the consequent redistribution of LTCC, which then led to enhanced phosphorylation after β_2-AR activation. Even though our HF model also considered detubulation, a reduced I_{CaL} phosphorylation by β_2-AR stimulation was observed, and β_1-AR was the primary factor responsible for I_{CaL} enhancement and EAD development.

4.5. Transmural gradients

The heterogeneous signaling and electrophysiological HF remodeling in the different transmural myocytes did not result in significant modulation of the different biomarkers in epicardial versus endocardial cells under β-AR stimulation. Unidimensional cable simulations were then performed to take into account additional factors such as intercellular coupling and electrical propagation in transmural gradients. The initial unicellular study revealed that the main differences between endocardial and epicardial myocytes observed in N conditions were maintained in HF, despite slight differences in the modulation of some biomarkers, such as CaTD-APD. Contractility seems to be therefore affected throughout the transmural wall in HF. An important difference was the larger propensity of endocardial cells to develop EADs due to their longer APD compared to epicardial cells. These cells might be the origin of triggered activity driven by elevated intracellular Ca^{2+} and the subsequent reactivation of I_{CaL} during a long AP.

The existence of transmural heterogeneities of repolarization, which are common in HF, can create a substrate for arrhythmias [56]. In contrast to the increase of transmural dispersion exerted by β-adrenergic stimulation in failing myocytes observed by Lang et al. [22], we observed only minimal β-adrenergic induced changes to TDR in HF. Moreover, β-AR stimulation TDR reduction benefits obtained in a N fiber following β_1-selective and non-selective stimulation were not present in HF. In most circumstances, however, TDR was greater in HF strands than in normal strands due to HF-induced electrophysiological remodeling and reduction in intracellular coupling that accompanies HF. In view of these results, it can be concluded that the increase of spatial gradients underlying the development of malignant ventricular arrhythmias in HF patients [57], are mainly due to ionic remodeling rather than to β-AR stimulation.

4.6. Clinical implications

Long-term β-blockers therapy is currently recommended for patients with HF to improve symptoms, reduce hospitalizations, and decrease mortality rate [10]. In addition to β-AR blockade, antagonist agents can have other properties such as antioxidant, anti-inflammatory, and vasodilating effects that contribute to their efficacy [13,58]. Based on the antiarrhythmic impact, which is the main cause of sudden cardiac death in the short term, this study suggests that β_1-AR blockade in HF, with selective or non-selective drugs, would be protective by reducing EAD events. A decrease in spatial repolarization gradients induced by β-blockade would also in principle be beneficial, but our results did not show such an effect in HF. Although previous experimental and computational investigations have suggested specific β_2-AR blockers to stop the detrimental effects of HF [22,35,46], we could not attribute arrhythmogenesis to β_2-ARs because of the diminished contribution in failing myocytes.

The benefits of β-AR antagonists have been observed in different cardiac pathologies (atrial fibrillation, long QT syndrome, myocardial infarction, among others) and explain their extended clinical use [59,60]. In atrial fibrillation, β-blockers are used for ventricular rate control and, despite not being the most effective antiarrhythmic therapy, they may be helpful for atrial fibrillation prevention under certain circumstances, such as reduced ejection fraction [60,61]. A recent exception found is the beneficial effect of β-AR stimulation restoration to reduce alternans in the postinfarction border zone reported by Tomek et al. [62]. Unlike the response of HF myocytes to β-AR stimulation, the antiarrhythmic effect of hyperinnervation after myocardial infarction could be due to a different remodeling in the border zone. But apart from the controversy, the aforementioned study, that combined experiments with simulations, also highlighted the potential of computational modeling in investigating mechanistic implications.

An alternative to β-blocker therapy would be the resensitization of the β-AR system to restore the beneficial effects of sympathetic stimulation, such as inotropic increase and reduction of TDR. Gene therapy, although in its early stages, can act directly on the known molecular targets that are altered in HF [63]. The observations of β-AR downregulation in HF led investigators to examine whether β_1-AR or β_2-AR overexpression was beneficial. Reactivation of β_1-ARs increases contractility initially but in the long term causes cardiac hypertrophy and fibrosis that contributes to lethal arrhythmias as well as cell death [38,64]. Whether β_2-AR overexpression improves cardiac function or not is still debated, but long term stimulation of β_2-ARs does not have cardiotoxic consequences [38,65]. Another approach proposed after

finding that elevated GRK activity led to dysfunctional β-AR in HF is GRK inhibition. At least in transgenic mice, this intervention has reduced ventricular dysfunction and is considered a promising therapy [38,66]. Similarly, the increase of inhibitor1 levels would also constitute a potential target to reduce PP1 inhibition function and ultimately increase SERCA activity. This last strategy, targeted to a specific signaling compartment, could increase therapeutic efficacy compared with drugs that affect global signaling, because different pathways and remodeling of the β-AR microdomains in myocytes determine PKA-phosphorylation and cardiac outcomes in HF [60,67].

4.7. Limitations

Our HF model reproduced the main changes in the β-AR system that have routinely been observed experimentally. Human data were included when available, but most data rely on experiments performed in animal models of HF. The comparison with the experimental cardiac response to sympathetic stimulation in human failing preparations highlights that additional experiments would be crucial to confirm our predictions. On the one hand, a consistent methodology for all experiments would facilitate the comparison of selective β-AR isoform stimulation results. The specificity of β-AR agonists is under debate and it would be interesting to know if their mechanism of action is equivalent to non-specific stimulation with β-blockers. On the other hand, despite the increasing research on β-AR stimulation in patients with HF, clinical trials do not provide enough information to validate signaling mechanisms. The scarcity of human data entails the dependence on other species at the molecular level, which might be the cause of the discrepancies between the mathematical model and experimental observations. β-AR system model with minor modifications was able to match human β-AR stimulation results with isoproterenol, but not the results obtained with isoform-specific stimulation. Additional parameters of the model should be readjusted to adapt the original canine β-AR model to human and for that, reliable electrophysiological responses would be required to confirm the results. Afterwards, additional signaling pathways such as muscarinic receptor interactions or additional proteins prone to PKA-phosphorylation could also help improve the model and the electrophysiological properties following sympathetic stimulation.

An additional limitation is the sympathetic stimulus used to activate β-AR with ligands, which has been simplified according to the available data and was sufficient for the purpose of the present study. Thus, sympathetic innervation was considered homogeneous instead of transmurally heterogeneous as some studies have shown [68] and agonist concentration was time-independent, fixed at saturated dose of maximal stimulation.

Despite some discrepancies with experimental observations, this work presents the most complete relation to date between β-AR signaling mechanisms and HF remodeling in single cardiac myocytes and transmural cellular strands. The mechanistic investigation of the effects in failing cardiomyocytes can help to predict the critical settings for lethal arrhythmias and guide in the search of potential molecular targets. However, additional experiments to support or refute model predictions would be valuable.

Disclosures

None.

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Appendix A. Supplementary data

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