

# Simulation and Mechanistic Investigation of the Arrhythmogenic Role of the Late Sodium Current in Human Heart Failure

Beatriz Trenor<sup>1\*</sup>, Karen Cardona<sup>1</sup>, Juan F. Gomez<sup>1</sup>, Sridharan Rajamani<sup>2</sup>, Jose M. Ferrero Jr<sup>1</sup>, Luiz Belardinelli<sup>2</sup>, Javier Saiz<sup>1</sup>

**1** Instituto de Investigación Interuniversitario en Bioingeniería y Tecnología Orientada al Ser Humano, Universitat Politècnica de València, Valencia, Spain, **2** Department of Biology, Gilead Sciences, Palo Alto, California, United States of America

## Abstract

Heart failure constitutes a major public health problem worldwide. The electrophysiological remodeling of failing hearts sets the stage for malignant arrhythmias, in which the role of the late Na<sup>+</sup> current ( $I_{NaL}$ ) is relevant and is currently under investigation. In this study we examined the role of  $I_{NaL}$  in the electrophysiological phenotype of ventricular myocytes, and its proarrhythmic effects in the failing heart. A model for cellular heart failure was proposed using a modified version of Grandi et al. model for human ventricular action potential that incorporates the formulation of  $I_{NaL}$ . A sensitivity analysis of the model was performed and simulations of the pathological electrical activity of the cell were conducted. The proposed model for the human  $I_{NaL}$  and the electrophysiological remodeling of myocytes from failing hearts accurately reproduce experimental observations. The sensitivity analysis of the modulation of electrophysiological parameters of myocytes from failing hearts due to ion channels remodeling, revealed a role for  $I_{NaL}$  in the prolongation of action potential duration (APD), triangulation of the shape of the AP, and changes in Ca<sup>2+</sup> transient. A mechanistic investigation of intracellular Na<sup>+</sup> accumulation and APD shortening with increasing frequency of stimulation of failing myocytes revealed a role for the Na<sup>+</sup>/K<sup>+</sup> pump, the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger and  $I_{NaL}$ . The results of the simulations also showed that in failing myocytes, the enhancement of  $I_{NaL}$  increased the reverse rate-dependent APD prolongation and the probability of initiating early afterdepolarizations. The electrophysiological remodeling of failing hearts and especially the enhancement of the  $I_{NaL}$  prolong APD and alter Ca<sup>2+</sup> transient facilitating the development of early afterdepolarizations. An enhanced  $I_{NaL}$  appears to be an important contributor to the electrophysiological phenotype and to the dysregulation of [Ca<sup>2+</sup>]<sub>i</sub> homeostasis of failing myocytes.

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**Competing Interests:** The authors have read the journal's policy and have the following conflicts: Dr. Belardinelli and Dr. Rajamani are employees of Gilead Sciences, the US company that markets Ranexa, and own stock and stock options. They are reimbursed for travel expenses to lecture on this subject. They have numerous patents related to late INA inhibitors, a list of which can be provided on request. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

\* E-mail: btrenor@eln.upv.es

## Introduction

Over 5 million persons in the United States suffer from heart failure (HF) and more than 250,000 die annually [1]. Patients with congestive HF are prone to develop complex ventricular tachyarrhythmias and some die suddenly [2]. Experimental studies conducted using animal models of HF have shown that ventricular arrhythmias are mainly due to non-reentrant mechanisms, most likely triggered activity based on afterdepolarizations [2].

Much attention has been paid to the understanding of the arrhythmogenic mechanisms induced by the structural, electrical, and metabolic remodeling of the failing heart. The electrophysiological remodeling of the failing heart has been well described (see [3,4] for review). Action potential (AP) prolongation, altered

Ca<sup>2+</sup> handling, as well as intracellular Na<sup>+</sup> ([Na<sup>+</sup>]<sub>i</sub>) accumulation have been established as the hallmark characteristics of myocytes and tissues isolated from failing human and canine hearts [5–8]. These alterations are closely related to arrhythmogenic mechanisms, such as early (EADs) and delayed (DADs) afterdepolarizations, observed in HF [9]. Functional remodeling of ion channels and pumps is the underlying cause for AP duration (APD) prolongation and altered intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) homeostasis. Downregulation of outward K<sup>+</sup> currents is the most consistent change in animal models and human HF [3,4,6,7,10]. Major changes in intracellular and sarcoplasmic reticulum (SR) Ca<sup>2+</sup> homeostasis are also associated with HF in several animal species, included human [9,11–13]. In myocytes from failing hearts [Na<sup>+</sup>]<sub>i</sub> concentration and Ca<sup>2+</sup> handling are closely linked; [Na<sup>+</sup>]<sub>i</sub> is

increased in failing ventricular myocytes from human and other animal species [3,4,9,14] and a prominent increase of the human late  $\text{Na}^+$  current ( $I_{\text{NaL}}$ ) has also been documented [15,16], and has been proposed as a therapeutic target [17–19]. Experimental studies have shown that the  $I_{\text{NaL}}$  is involved in the generation of EADs in myocytes [17,18] and life-threatening arrhythmias, such as torsade de pointes (TdP) [20], especially under conditions of reduced repolarization reserve in several animal species [21–23]. Other changes in biomarkers for arrhythmic risk such as the increase in the reverse rate-dependent APD prolongation [20,23,24] have been attributed to an increase in  $I_{\text{NaL}}$  concomitant with inhibition of outward  $\text{K}^+$  currents.

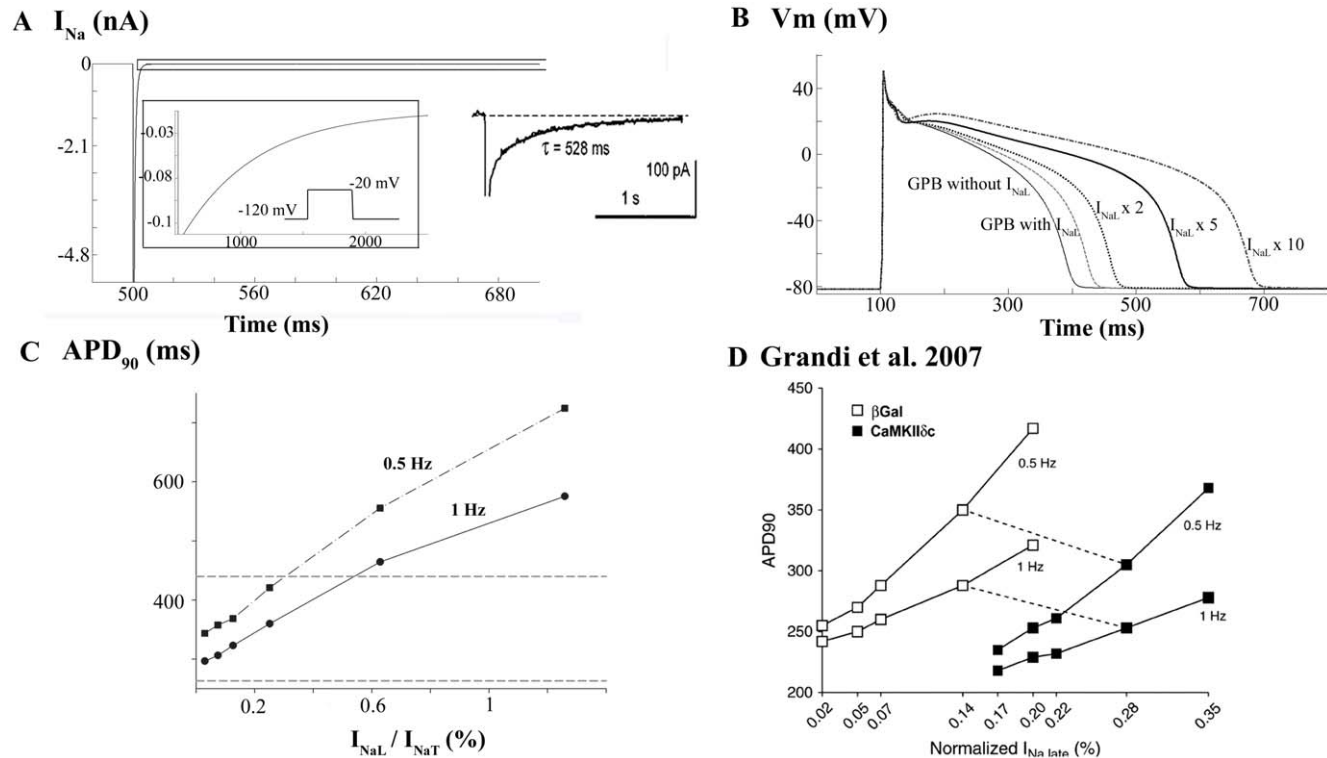
Thus, the goal of our study was to analyze, using computer simulations, the role of the  $I_{\text{NaL}}$  in the setting of human HF. A mathematical model of human HF is proposed at cellular level using a modified version of the Grandi et al. model (from herein referred to as the GPB model) [25] of endocardial AP, in which a new formulation of the  $I_{\text{NaL}}$  is included. The sensitivity analysis performed for the HF model, as well as the simulations of the rate-dependence of APD and EAD generation revealed that the mechanisms underlying the arrhythmogenic processes taking place in HF conditions, from a theoretical point of view, include an enhanced  $I_{\text{NaL}}$ .

## Results

### Effects of $I_{\text{NaL}}$ on human AP

Figure 1A shows the generation of  $I_{\text{NaL}}$  trace using a voltage clamp protocol similar to that of Maltsev et al. [26] in ventricular

mid-myocardial myocytes at room temperature. The simulated  $I_{\text{NaL}}$  had similar time evolution and amplitude to the experimental findings. Panels B and C show the effects of this current on AP and APD at 90% of repolarization ( $\text{APD}_{90}$ ), respectively. The inclusion of  $I_{\text{NaL}}$  in the GPB model slightly prolongs steady-state APD at 1 Hz (see Figure 1B). APD remains within the physiological range for human endocardial myocytes [8,27,28]. By increasing the magnitude of  $I_{\text{NaL}}$  by a factor of two-, five- or ten-fold, we obtained  $\text{APD}_{90}$  prolongations of 11%, 44% and 78%, respectively. Experimental recordings of monophasic APs (MAPs) under the effects of veratridine, an enhancer of  $I_{\text{NaL}}$ , show similar effects on APD prolongation in rabbit myocytes [29]. We determined the sensitivity of  $\text{APD}_{90}$  to  $I_{\text{NaL}}$  amplitude at different stimulation rates. As shown in Figure 1C,  $I_{\text{NaL}}/I_{\text{NaT}}$  ratios, where  $I_{\text{NaT}}$  is the transient peak  $I_{\text{Na}}$ , were varied from 0.0298% to 1.26% and  $\text{APD}_{90}$  significantly increased according to the magnitude of  $I_{\text{NaL}}$ . Furthermore, the sensitivity of  $\text{APD}_{90}$  increased with the slowing of the stimulation rate (compare 1 Hz and 0.5 Hz). Similar simulations were carried out in a rabbit model of AP [30]. As shown in Figure 1D, the difference in sensitivity of  $\text{APD}_{90}$  to changes in frequency of stimulation (0.5 and 1 Hz) was also observed. Note that the morphology of the curves in Figure 1D is different to our results, as different models for  $I_{\text{NaL}}$  and different AP models were used. The ratio chosen in our model of 0.12% yields an  $\text{APD}_{90}$  that is within the physiological range for 1 Hz indicated by the discontinuous lines.



**Figure 1. Time course of late  $\text{Na}^+$  current and its effects on AP.** A: Simulation of late  $\text{Na}^+$  current ( $I_{\text{NaL}}$ ) using a voltage clamp protocol similar to that of the experimental measurements obtained by Maltsev et al. [26] (shown in the right inset) in ventricular myocytes at room temperature. The left inset shows an expanded view of the current between 480 ms and 2500 ms. B: Simulated action potentials (APs) at 1-Hz pacing rate using the GPB model, the GPB model modified with control  $I_{\text{NaL}}$  and  $I_{\text{NaL}}$  enhanced 2-fold, 5-fold and 10-fold. C:  $\text{APD}_{90}$  sensitivity to the  $I_{\text{NaL}}$  amplitude. APs were simulated at 0.5-Hz (square symbols) and 1-Hz (circle symbols) pacing rate by varying  $I_{\text{NaL}}/I_{\text{NaT}}$  ratio from 0.0298% to 1.26%. The range of experimental APD at 90% repolarization ( $\text{APD}_{90}$ ) for human is represented by the two discontinuous lines. D:  $\text{APD}_{90}$  sensitivity to the  $I_{\text{NaL}}$  amplitude (open symbols) taken from Grandi et al. [30] who used a rabbit model. doi:10.1371/journal.pone.0032659.g001

## Heart Failure model and sensitivity analysis

APs and  $[Ca^{2+}]_i$  transients were simulated under conditions of HF. Figure 2 shows APs (panel A),  $[Ca^{2+}]_i$  transients (panel B) and  $Na^+/Ca^{2+}$  exchanger (NCX) function ( $I_{NCX}$ ) (panel C) of failing (light line) and nonfailing (dark line) ventricular myocytes after achieving steady-state conditions for a stimulation rate of 1 Hz. Our results showed an  $APD_{90}$  prolongation of 24% in failing myocytes versus normal ones, as well as a 18% prolongation in  $APD_{50}$ , so that triangulation ( $APD_{90}-APD_{50}$ ) increased by 43% under HF conditions. The experimental observations shown in the inset of Figure 2 (panel A) taken from reference [5], show the variability of the APD in failing myocytes from human hearts, which falls within the simulated values of APD observed.

Figure 2B depicts an altered  $[Ca^{2+}]_i$  transient under HF conditions, as has been documented experimentally and is shown in the inset [31]. Indeed, diastolic  $[Ca^{2+}]_i$  is slightly increased, whereas peak systolic  $[Ca^{2+}]_i$  is reduced to 41% of the one observed during normal conditions. An additional reported feature of the  $[Ca^{2+}]_i$  transient of a failing myocyte is its slow decay. In our simulations  $Ca^{2+}$  transient decay, quantified as the time needed from the peak value to reach 10% of the transient amplitude ( $\delta_{Ca}$  decay), yielded 630 ms and 380 ms in the failing and nonfailing myocytes, respectively. Finally, Figure 2C illustrates the changes in  $I_{NCX}$  during HF, mainly a shift in the time of the reversal potential for the NCX ( $t_{NCXRP}$ ) of 20 ms. Similar shifts in  $t_{NCXRP}$  have been reported in experimental studies [31].

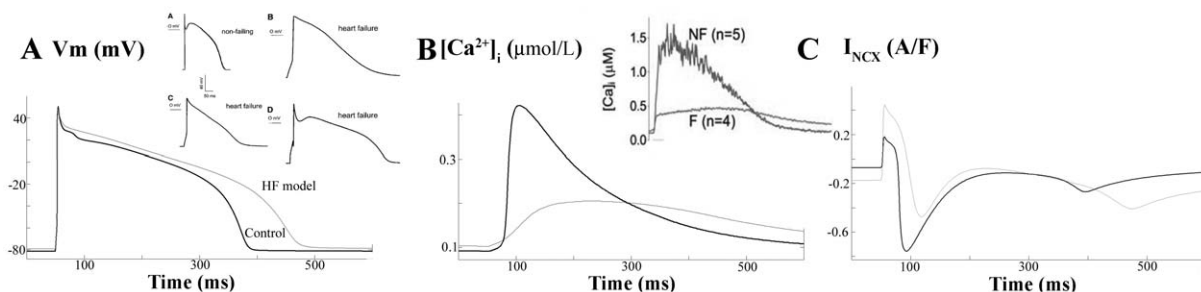
Changes in  $[Na^+]_i$  and  $[Ca^{2+}]_i$  levels under HF conditions at various stimulation rates were also investigated using a staircase protocol described in the methods section and described previously [32–34]. Figure 3A shows that diastolic  $[Ca^{2+}]_i$  level is higher in HF than in normal conditions and systolic level is always higher in normal conditions than in HF, as reported experimentally [5,35,36]. The impact of the variability of ion channel remodeling on these results are presented in Figures S4, S5, S6, S7.

Another feature of myocytes from failing hearts is the high concentration of  $[Na^+]_i$  regardless of the stimulation rate, as observed in Figure 3B and experimentally reported by Pieske et al. [33] in failing human myocardial cells. However, as acknowledged by Pieske et al. [33], it should be noted that the absolute  $[Na^+]_i$  values in their experimental work were overestimated in normal and failing hearts. The impact of the variability of ion channel remodeling on these results are presented in Figures S8 and S9.

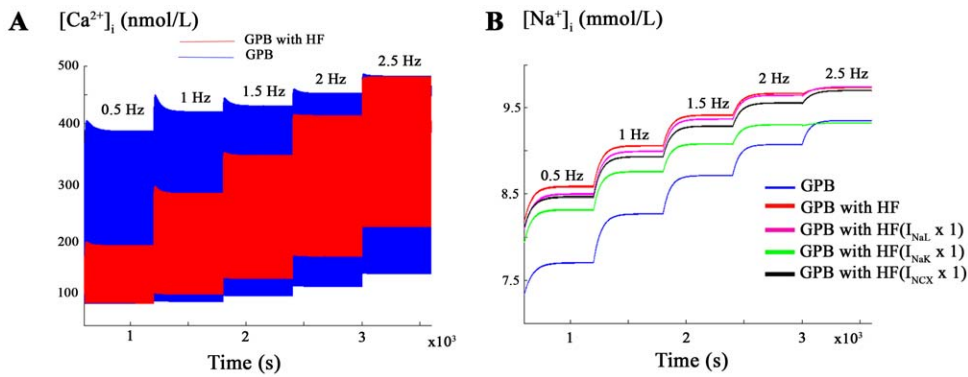
To analyze the mechanisms responsible for  $[Na^+]_i$  accumulation with frequency in failing myocytes, the above pacing protocol was applied for a) normal conditions (blue), b) HF conditions (red), c) HF with no  $I_{NaL}$  upregulation (purple), d) HF with no  $Na^+/K^+$  pump current ( $I_{NaK}$ ) downregulation (green) and e) HF with no  $I_{NCX}$  upregulation (black). From the results obtained in Figure 3B,

it can be deduced that  $[Na^+]_i$  accumulation is mainly driven in HF by alterations in  $I_{NaK}$ , and much less by the  $I_{NCX}$  and  $I_{NaL}$ .

Our basic HF model, as described in Figure 2 and 3, reproduced the main changes in the electrophysiological (EP) characteristics of myocytes from failing hearts. However, the experimentally reported ranges for EP changes, as well as the magnitude of ion channels, transporters and respective current remodeling, vary substantially for different experimental settings and HF stages. Hence, we performed a sensitivity analysis to assess the impact of the main ionic parameters remodeled in HF on the described EP characteristics. Figure 4A highlights the role of  $I_{NaL}$  and  $I_{NaK}$  in  $APD_{90}$  under conditions of HF. When  $I_{NaL}$  undergoes a 2-fold increase (i.e. doubling) with respect to the basic HF increase, APD is prolonged 22% and no change at all in this current leads to a shortening of the  $APD_{90}$  by 10% with respect to basic HF conditions. This result is in agreement with experimental recordings in failing human myocytes, where  $I_{NaL}$  has a crucial role in APD [19]. Similarly, the downregulation of  $Na^+/K^+$  pump in HF has a relevant effect on  $APD_{90}$  shortening, and further reduction of the current leads to a decrease of 22% with respect to HF  $APD_{90}$  value, which is in agreement with experimental observations [37]. Figure 4B reveals the importance of  $I_{NaK}$ ,  $I_{NCX}$ , the background  $Ca^{2+}$  current ( $I_{Cab}$ ), the leak  $Ca^{2+}$  current ( $I_{leak}$ ), and  $I_{NaL}$  in determining the value of peak systolic  $[Ca^{2+}]_i$  during HF. Regarding the regulation of  $[Na^+]_i$  value in failing myocytes, Figure 4C shows the important role of  $I_{NaK}$ ,  $I_{Cab}$ , the inward rectifier  $K^+$  current ( $I_{K1}$ ),  $I_{NCX}$  and  $I_{NaL}$ . To summarize the sensitivity of some of the EP characteristics (1<sup>st</sup> column) during HF to the altered ionic parameters (1<sup>st</sup> row), Figure 5 shows the relative sensitivity normalized to the maximum sensitivity for that particular characteristic, as described in the methods section. The positive and negative signs indicate whether the change of the ionic current and the HF EP characteristic follow the same or inverse tendency, respectively. Percentages in each box indicate the maximum absolute sensitivity of the EP parameter correspondent to that row for all ionic properties. From this sensitivity analysis it could be deduced that APD was particularly sensitive to  $I_{NaL}$  and to  $I_{NaK}$  (green and burgundy colors in rows 1 and 2). Furthermore,  $I_{K1}$ ,  $I_{NCX}$  and  $I_{NaK}$  have an important effect on AP triangulation. The main features of  $Ca^{2+}$  transient in HF (3 medium rows) were mainly influenced in this order by  $I_{NaK}$ , SERCA function,  $I_{NCX}$ ,  $I_{leak}$ ,  $I_{Cab}$ , and  $I_{NaL}$ . The SR  $Ca^{2+}$  concentration ( $[Ca^{2+}]_{SR}$ ) is also influenced by other currents but in this case  $I_{leak}$  becomes more important than  $I_{NCX}$ .  $[Na^+]_i$  is mainly regulated by  $I_{NaK}$ ,  $I_{Cab}$ ,  $I_{K1}$ ,  $I_{NCX}$  and  $I_{NaL}$ . Finally,  $t_{NCXRP}$  is mainly modulated by the SR  $Ca^{2+}$ -ATPase activity ( $I_{SERCA}$ ). The absolute effects of small changes ( $\pm 15\%$ ) in the ionic remodeling of the basic HF model on the main results of our simulations are



**Figure 2. Electrophysiological changes in heart failure.** Simulated APs (panel A),  $[Ca^{2+}]_i$  transients (panel B), and  $Na^+/Ca^{2+}$  exchanger (NCX) activity (panel C) at 1-Hz pacing rate in control (dark line) and in heart failure (HF) conditions (light line). The insets show experimental recordings of Priebe and Beuckelmann et al. [5] (panel A) and Weber et al. [31] (panel B). doi:10.1371/journal.pone.0032659.g002



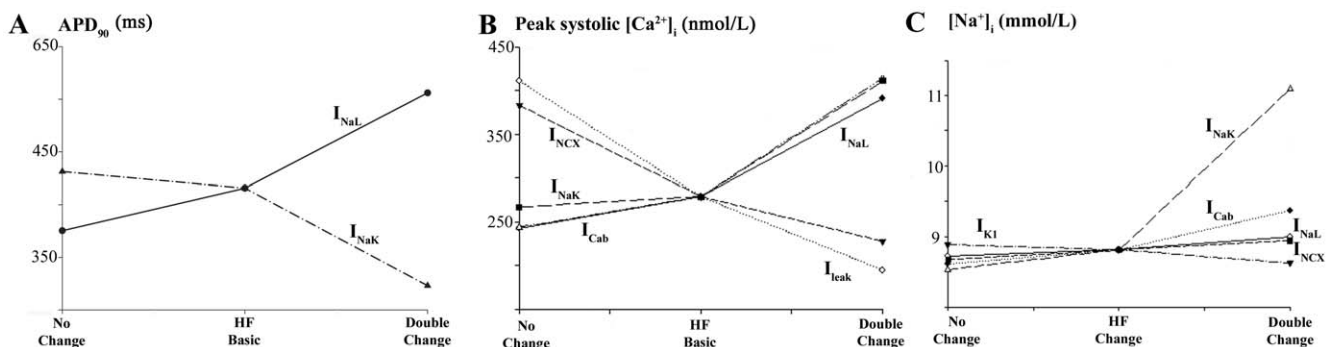
**Figure 3.  $[Ca^{2+}]_i$  and  $[Na^+]_i$  changes with increasing frequency in HF.** Influence of the stimulation rate on  $[Ca^{2+}]_i$  (panel A) and  $[Na^+]_i$  in nmol/L (panel B) determined using the staircase protocol. The simulations were performed using the modified GPB model with  $I_{NaL}$  incorporated in normal conditions (blue) and the modified GPB model incorporating HF conditions (red). In panel B, conditions of HF without  $I_{NaL}$ ,  $I_{NaK}$  or  $I_{NCX}$  remodeling are also shown.  
doi:10.1371/journal.pone.0032659.g003

shown in Figures S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, and S11. These absolute effects are in agreement with the relative role of the ionic parameters described above. We have especially focused on the sensitivity of  $APD_{90}$  in failing myocytes to  $I_{NaL}/I_{NaT}$  ratio, as shown in Figure S1. In this figure, the values of  $I_{NaL}/I_{NaT}$  ratios for 1 Hz in the modified the GPB model and in our basic HF model are indicated by arrows and are within experimental ranges (indicated by blue dotted lines in normal myocytes and by red discontinuous lines in failing myocytes, respectively). Changes in the selected ratios affect the  $APD_{90}$  values of failing and normal myocytes, which stay within experimental ranges provided that the changes of these ratios are not very large.

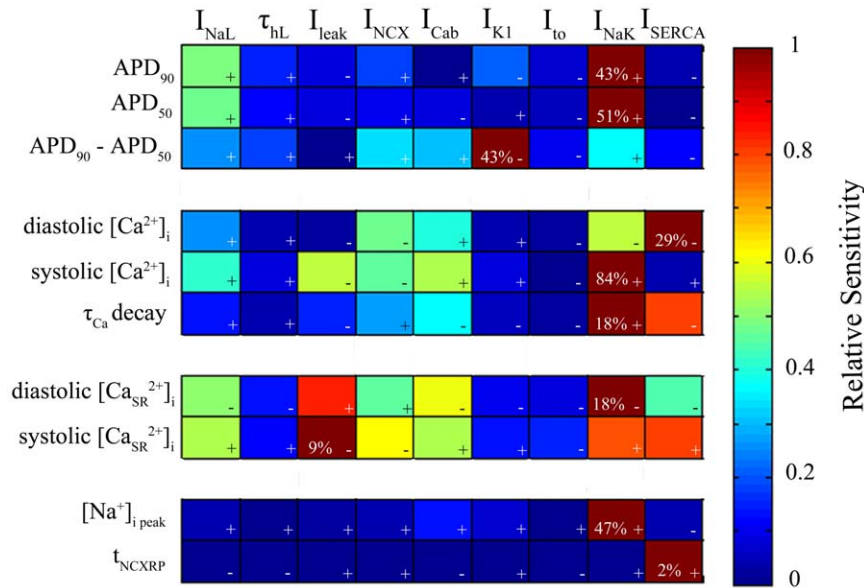
#### APD rate-dependence and the role of $I_{NaL}$ in HF

The role of  $I_{NaL}$  in steady-state  $APD_{90}$  rate-dependence during HF was analyzed. An enhanced reverse frequency-dependence of  $APD_{90}$  is considered to be a major proarrhythmic risk (see [38] for review). We performed simulations and measured  $APD_{90}$  under normal and HF conditions for different magnitudes of  $I_{NaL}$  activation (see Figure 6A). Also 40% and 60% inhibition of the rapid component of the delayed rectifier  $K^+$  current ( $I_{Kr}$ ) in failing myocytes were simulated with different degrees of  $I_{NaL}$  enhancement (see Figure 6B). Indeed, the concomitant alteration of  $I_{NaL}$  and  $I_{Kr}$  has been reported to have important effects on arrhythmogenicity [20–24]. An important result obtained in our

simulations, as shown in Figure 6A, is the reverse rate-dependence effect on  $APD_{90}$  exerted by  $I_{NaL}$  enhancement for normal conditions (compare circles with stars). The rate-dependence yielded 110 and 190 ms maximal  $APD_{90}$  prolongation in normal conditions versus  $I_{NaL}$  increased by 4-fold, respectively. Similar observations were reported by others [20,24] in rabbit ventricular myocytes under the effects of  $I_{NaL}$  activators, as well as in isolated perfused rabbit heart [22]. When considering HF conditions (squares), the rate-dependence is also increased with respect to normal conditions. The  $\Delta APD$  value is very similar to the value obtained with  $I_{NaL}$  enhanced (stars). This implies that  $I_{NaL}$  might be the main driver of the increased reverse rate-dependent prolongation of APD. Additionally, greater magnitudes of  $I_{NaL}$  (black triangles) are associated with greater increase in the reverse rate-dependency of APD of failing myocytes. Experimental studies [7] have also documented the increase of APD rate-dependence in human failing epicardial myocytes, as depicted in the inset. Simulations of APD rate-dependence in HF caused by small changes ( $\pm 15\%$ ) in the ionic remodeling of the basic HF model (see Figures S2, S3, S4, S5, S6, S7, S8, S9, S10, and S11) to take into account the experimental variability on electrical remodeling during HF were also performed. The results do not change significantly with respect to the basic HF model, except for  $I_{NaK}$  and  $I_{NaL}$ , which have an important effect on  $APD_{90}$ , as predicted by the sensitivity analysis shown in Figure 5.



**Figure 4. Sensitivity of electrophysiological parameters to changes in ionic current properties.** Changes in  $APD_{90}$  (panel A), peak systolic  $[Ca^{2+}]_i$  (panel B), and  $[Na^+]_i$  (panel C) with changes in  $I_{NaL}$ ,  $I_{NaK}$ ,  $I_{leak}$ ,  $I_{NCX}$ ,  $I_{K1}$  and  $I_{Cab}$ , as labeled next to each curve. Axis x represents the simulation conditions; for “HF Conditions” the remodeling of the basic HF model is considered, for “No Change” the labeled current is unchanged as it is in the GPB model, for “Double Change” the labeled current undergoes a double change with respect to the change exerted in “HF conditions.”  
doi:10.1371/journal.pone.0032659.g004



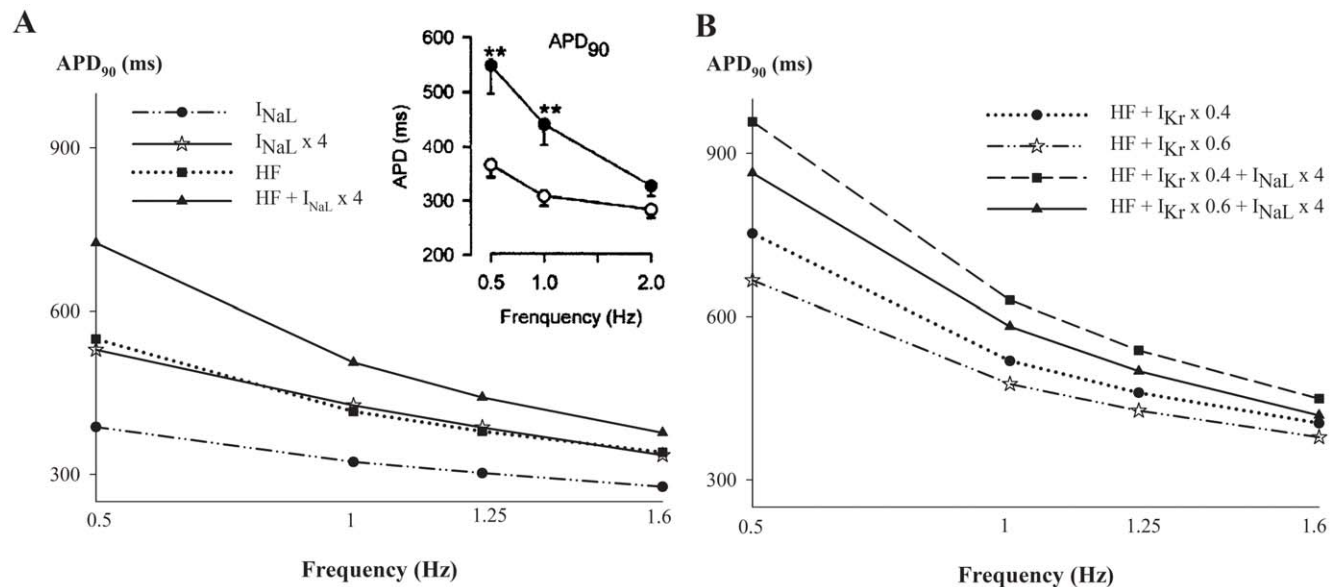
**Figure 5. Relative sensitivities of the electrophysiological parameters to changes in ionic current properties.** Dark blue color indicates lack of dependency between the ionic property (1<sup>st</sup> row) and the EP parameter (1<sup>st</sup> column), and dark red color indicates strong direct (+) dependency or inverse dependency (-). Percents in each box indicate the maximum absolute sensitivity of the EP parameter correspondent to that row for all ionic properties.

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Figure 6B summarizes the concomitant contribution of  $I_{NaL}$  enhancement and  $I_{Kr}$  inhibition in the reverse rate-dependency of APD, shown in various experimental studies [20,24], in failing myocytes.

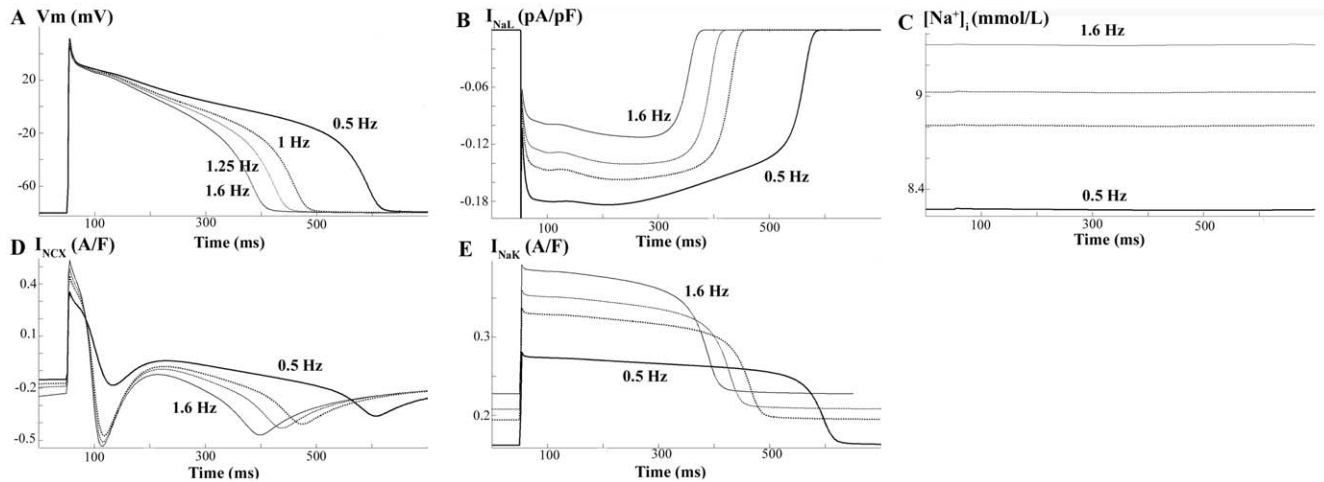
To analyze the ionic mechanisms responsible for steady-state APD rate-dependence in failing myocytes, we determined the behavior of several ionic currents ( $I_{NaL}$ ,  $I_{NCX}$  and  $I_{NaK}$ ) and  $[Na^+]_i$  at different frequencies of stimulation, as depicted in Figure 7. Our

results show that the shortening of the APD with increased frequency in HF is explained on the one hand by the decrease in  $I_{NaL}$ , indeed faster rates lead to an incomplete recovery from inactivation of this current [39–41], and on the other hand by  $[Na^+]_i$  accumulation leading to an increase of  $I_{NaK}$ . The role of  $I_{NCX}$  on APD is not evident as both outward and inward modes are enhanced. Although several experimental studies suggest the direct involvement of this current on APD shortening with



**Figure 6. Role of  $I_{NaL}$  in APD rate-dependence in HF and reduced repolarization reserve.** Simulated APD dependence on stimulation frequency for normal (circles in panel A) and HF (squares in panel A) conditions and in the presence of enhanced  $I_{NaL}$  in normal (stars in panel A) and HF (triangles in panel A) conditions. The inset shows experimental results of Li et al. [7]. Panel B, depicts the APD rate-dependence for HF combined with different degrees of  $I_{Kr}$  inhibition and  $I_{NaL}$  enhancement.

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**Figure 7. Mechanisms for APD rate-dependence in HF.** Simulated APs and ionic currents at different stimulating frequencies (0.5, 1, 1.25 and 1.6 Hz) under HF conditions.

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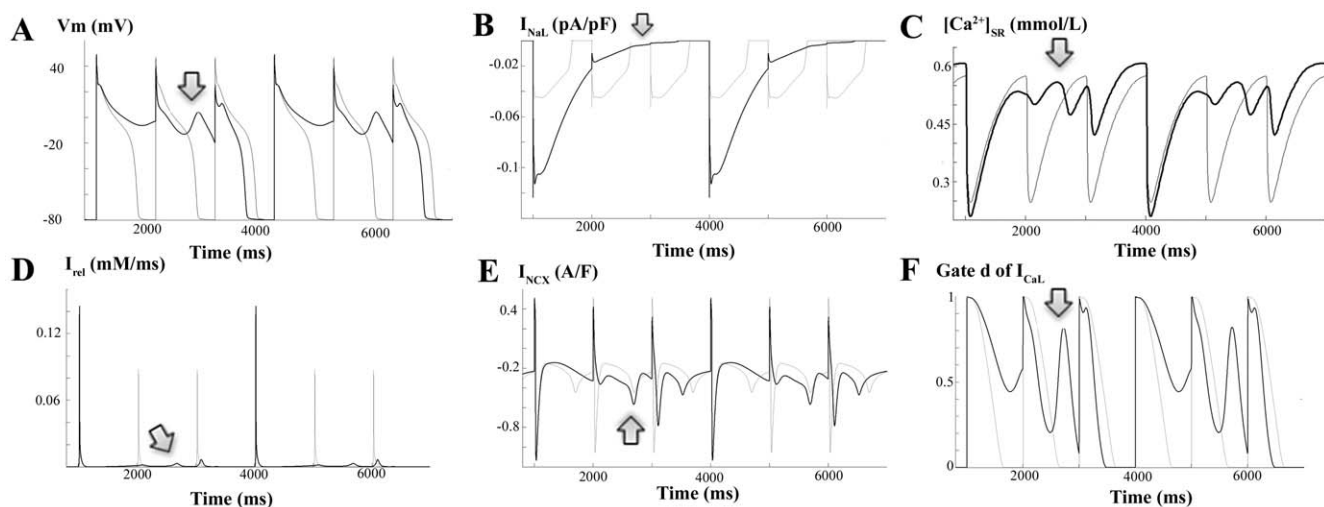
frequency, as will be discussed later, its implication in our simulations is not evidenced. Indeed, when we simulated the cell at high frequency (1.6 Hz) but clamped  $[Na^+]_i$  value to the lower  $[Na^+]_i$  value corresponding to the low frequency (0.5 Hz), we observed a decrease in  $APD_{90}$  with respect to  $APD_{90}$  at low frequency, concomitant with a decrease and increase of the outward and inward modes of NCX activity, respectively (results not shown). These changes in  $I_{NCX}$  should lead to a longer  $APD_{90}$ , instead of shorter, suggesting that  $I_{NCX}$  is not determinant in APD rate-dependence in the present model.

#### Arrhythmogenic effects of $I_{NaL}$

Under basic HF conditions, APD prolongation favors the occurrence of EADs. We were therefore interested in determining the role of  $I_{NaL}$  in the repolarization abnormalities of human failing hearts. We stimulated the myocyte at 1 Hz and simulated conditions of low repolarization reserve prone to EADs generation.

Under HF conditions using the basic HF model,  $I_{Kr}$  was reduced by 50% and the  $Ca^{2+}$  current ( $I_{CaL}$ ) was increased by 30%. Figure 8A shows EADs (dark line) when  $I_{NaL}$  was doubled, whereas APs displayed no EADs when  $I_{NaL}$  was normal (light line). The impact of the variability in ion channel remodeling on EADs generation can be observed in Figures S10 and S11. The mechanisms by which EADs arise are dictated by a very delicate balance of ionic currents during AP plateau. Slight changes in this balance can suppress EADs, as occurs in the case when all ionic parameters are reduced in 15% with respect to the basic HF model (see Figure S11).

Figure 8 depicts APs and ionic currents to provide insight into the mechanisms by which enhancement of  $I_{NaL}$  indirectly triggers EADs [17]. The APD prolongation (panel A) caused by enhancement of  $I_{NaL}$  (panel B) leads to the reactivation of the activation gate d (panel F) of the  $I_{CaL}$ , which triggers the EAD, as was suggested by January et al. [42]. However, the important contribution of the altered  $Ca^{2+}$  transient should also be taken into



**Figure 8. Mechanisms for early afterdepolarizations with enhanced  $I_{NaL}$ .** Simulated APs and ionic currents at a stimulating rate of 1 Hz for HF conditions, 50% inhibition of  $I_{Kr}$  and 30% increase of  $I_{CaL}$ . Panel A shows early afterdepolarizations (EADs; dark line) when  $I_{NaL}$  was doubled and APs with no EADs when  $I_{NaL}$  was normal (light line). The temporal evolutions of  $I_{NaL}$  (panel B),  $I_{rel}$  (panel D),  $I_{CaL}$  (panel C), NCX activity (panel E), and activation gate of  $I_{CaL}$  are also depicted when  $I_{NaL}$  was doubled (dark line) and when  $I_{NaL}$  was normal (light line).

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consideration. Indeed, the APD prolongation caused by  $I_{NaL}$  enhancement alters  $Ca^{2+}$  handling. As observed in panel C,  $[Ca^{2+}]_{SR}$  remains elevated and provokes the trigger of a small spontaneous release (panel D, arrow) just before the EAD arises (panel A, arrow). NCX (panel E) extrudes  $Ca^{2+}$  operating in the inward mode, which contributes to membrane potential increase. This would contribute to  $I_{CaL}$  reactivation which triggers the EAD. The involvement of these currents is also shown in Figures S12, S13, S14, and S15, as a partial block of SR  $Ca^{2+}$  release ( $I_{rel}$ ) or  $I_{NCX}$  suppresses the EADs.

## Discussion

### Major Findings

In this article, one of the most recent and detailed AP model for human ventricular myocytes, the GPB model, was modified to simulate HF functional remodeling. The  $I_{NaL}$  was formulated and introduced in the model on the basis of human ventricular voltage-clamp data. An important novelty of the study is the sensitivity analysis of the modulation of the main EP characteristics due to ion channel and transporters remodeling, highlighting the role of  $I_{NaL}$  in the changes of APD, triangulation and  $Ca^{2+}$  transient under HF conditions. A mechanistic investigation of  $[Na^+]_i$  accumulation and APD shortening with increasing stimulation frequency in failing myocytes, revealed the roles of  $I_{NaK}$ ,  $I_{NCX}$  and  $I_{NaL}$ . Finally, the arrhythmogenic effects of increased  $I_{NaL}$  were investigated. Our results showed the important role of  $I_{NaL}$  in APD reverse rate-dependence and EAD generation in failing myocytes, representing the first realistic simulation study on this issue, which complements experimental studies and sheds light into the ionic mechanisms responsible for HF phenotype.

### Simulation of heart failure

Recently, O'Hara et al. [43] published a new AP model for human ventricular myocytes; however, in the present study the GPB model was chosen because its behavior was adequate to analyze the changes exerted in HF in APD,  $Ca^{2+}$  transient, the APD rate-dependence and EADs generation. Nevertheless future studies comparing the behavior of both models in pathological situations should be conducted. The GPB model was developed using human voltage-clamp data, and validated against recent experimental results [25]. In the present study, we demonstrate for the first time that the GPB model, subject to ion channel and transporter remodeling, is also appropriate to reproduce the electrical behavior of failing myocytes.

A large body of experimental research has focused on the functional remodeling of failing hearts [3,4,6,7,10,11,14]. However, little has been done in this field from a theoretical perspective. One of the first human ventricular AP models, including HF remodeling, was formulated by Priebe and Beuckelmann in 1998 [5]. However,  $I_{NaL}$  was not considered, and the formulation of  $Ca^{2+}$  handling was not as realistic as it is in the GPB model. This represents an important limitation for HF, when  $Ca^{2+}$  transient undergoes critical changes. Later, in 2005, Zhang et al. [44] modified the human ten Tusscher model [45] to simulate HF. In the latter model,  $I_{NaL}$  was considered, but with experimental data derived using dog ventricular myocytes [46], and remodeling was derived using experimental data from multiple species. However, controversy about the changes introduced in their model for  $I_{NaT}$ , the slow component of the delayed rectifier  $K^+$  current ( $I_{Ks}$ ) or  $I_{CaL}$  exists for human failing myocytes [2–4]. Other authors have simulated ion remodeling of myocytes from canine and rabbit failing hearts [12,47] that reproduce the APD and  $Ca^{2+}$  transient changes associated with

HF. Our model of basic HF not only reproduces experimental observations on APD prolongation and  $Ca^{2+}$  transients alterations [5,7,11,31], but also the APD,  $[Ca^{2+}]_i$  and  $[Na^+]_i$  rate-dependence reported in failing myocytes [33,35,36]. The ionic model of ventricular HF presented in this study is not only based on recent human experimental data, but also takes into account the variability documented in EP studies using sensitivity analysis. Sensitivity analysis also elucidates the ionic mechanisms responsible for the main changes in the EP characteristics of HF. Previous sensitivity analysis have been performed using the human ten Tusscher et al. [48] AP model [32], a modified version of the GPB model [34] and O'Hara et al. model [43], as well as rabbit [48] AP model. In these studies, the authors analyzed the effects of the physiological variability of the main ionic properties on several electrophysiological characteristics and biomarkers for arrhythmic risk, under normal physiological conditions. The main similarities of our results in HF with their studies on normal myocytes, are the importance of  $I_{NaK}$  [32,34] and  $I_{NaL}$  [43] in regulating APD<sub>90</sub>, and  $I_{K1}$  [32,34] and  $I_{NaK}$  in regulating AP triangulation. The variability of  $I_{CaL}$  and  $I_{Kr}$  has also an important effect in their studies but was not considered in HF, as these currents are not remodeled. Also the impact of  $I_{NaK}$  and  $I_{NCX}$  was important on  $Ca^{2+}$  transients under normal conditions [32,34].

Regarding the variability of functional remodeling in HF, it is worth noting that experimental measurements reported in literature have been conducted in different stages of HF (see [3,4] for review). In the present study, ionic currents are within experimental ranges, but do not take into account differences in various stages of HF. Different combinations of changes were tested in the sensitivity analysis in order to determine the relative effects on the EP characteristics in HF (Figure 5) and the absolute effects of small changes ( $\pm 15\%$ ) in ionic parameters on APD-rate-dependence (Figures S2 and S3),  $[Ca^{2+}]_i$  transients (Figures S4, S5, S6, and S7),  $[Na^+]_i$  values (Figures S8 and S9) and EADs generation (Figures S10 and S11). It should be noted that the results of the present sensitivity analysis are dependent on the values chosen for the ionic parameters in the basic HF model, as the percents of change also depend on these chosen values. The results suggest that the downregulation of the  $Na^+/K^+$  pump has an important role in the electrophysiological and  $Ca^{2+}$  transient changes in HF, highlighting the need for new experimental data. Electrophysiological studies [49] conducted in failing human hearts revealed increased  $Ca^{2+}$  transients under the effects of strophanthidin, a  $Na^+/K^+$  pump blocker, which is in agreement with our results. However, little is known about the effects of  $Na^+/K^+$  pump activity on APD in HF. Experiments carried out on hypertrophic rat hearts by Levi et al. [37] showed that the application of ouabain, a  $Na^+/K^+$  pump blocker, resulted in APD shortening, which was caused by  $[Na^+]_i$  accumulation and the subsequent increase in the reverse mode of the NCX. Similar studies should be performed using human failing hearts to determine the role of the effects of  $Na^+/K^+$  pump on APD. Also the  $[Na^+]_i$  accumulation with increasing frequency during HF appears to be mainly due to the downregulation of this pump, and a small contribution of  $I_{NaL}$  and  $I_{NCX}$  upregulation (see Figure 3B). Experimental studies reporting  $[Na^+]_i$  accumulation in human HF [33] or animal species [35,50] suggest that  $I_{NaK}$  downregulation,  $I_{NaL}$  and  $I_{NCX}$  upregulation, and altered activity of the  $Na^+/H^+$  exchanger (not included in the GPB model), might be involved. However, no experiments have been performed to in human failing myocytes to clarify the responsible mechanisms. Other simulation studies [30,51] using the Shannon et al. rabbit AP model [52] have also reported the small and important influence

of  $I_{NaL}$  and  $I_{NaK}$ , respectively, on  $[Na^+]_i$  accumulation with frequency under pathological situations other than HF.

Our results also reveal the role of  $I_{NaK}$  in APD rate-dependence as stated by Carmeliet [53] and by Eisner et al. [54] in their review on AP rate adaptation as well as shown in the theoretical and experimental findings by Pueyo et al. [55]. Additional simulation studies using different human AP models have also demonstrated this fact in normal conditions [32,43]. Although O'Hara et al. [43] and Carro et al. [34] human AP models exhibit an improved behavior in the fast phase of APD rate adaptation than the GPB model, our study focused on the steady-state APD rate-dependence, where the GPB model is valid except for low frequencies [43]. Also Faber and Rudy [56] showed that APD shortening at high rates in a guinea pig AP model was due to the increase of the outward NCX activity. This observation is also consistent with experiments conducted on myocytes from human failing hearts [49], where increases in  $[Na^+]_i$  led to APD shortening provoked by an increase of the reverse mode of  $I_{NCX}$ . However, the GPB model exhibits a much shorter outward mode of NCX than the Faber and Rudy model. Thus its contribution to AP repolarization is not evident in the present study. Furthermore, in failing human myocytes,  $Ca^{2+}$  influx via NCX is prominent during most of the plateau phase [31]. Thus changes in  $I_{NCX}$  formulation of the GPB model should be addressed to better understand the role of this current in HF APD rate-dependence. Also new experiments in failing human myocytes demonstrating the role of  $I_{NaK}$  and  $I_{NCX}$  in APD rate-dependence would be of great interest. This finding also suggests that the functional increase of these outward currents caused by  $[Na^+]_i$  accumulation may limit AP prolongation in HF, counterbalancing the increase of  $I_{NaL}$ .

Our results also highlight the pivotal role of  $I_{NaL}$  in the changes of EP characteristics in HF, which will be discussed in the following section. Finally, another mechanistic effect of relative importance in HF, revealed by the simulations of the present study is the increase of AP triangulation associated with  $I_{K1}$  downregulation. Similar observations have been reported in ventricular myocytes from normal dogs when  $I_{K1}$  was reduced with barium [57] and in the sensitivity analysis performed by Romero et al. [32] and Carro et al. [34] in normal physiological conditions.

### Important role of $I_{NaL}$ during heart failure and clinical implications

The pivotal role of  $I_{NaL}$  in the changes of EP characteristics and in arrhythmogenesis in HF has been uncovered in the present work. The formulation of the  $I_{NaL}$  model and its introduction in the GPB model was essential to draw our conclusions. Previous formulations have been proposed to model the behavior of  $I_{NaL}$  for different animal species using the Hodgkin-Huxley or Markov formalisms [19,20,30,46,58,59]. Noble and colleagues [59] in 1998 formulated the  $I_{NaL}$  for guinea pig by adding to their AP model a  $Na^+$  current with reduced conductance and slow inactivation. Similarly, other Hodgkin-Huxley formulations have been proposed for rabbit [20] and dog [19,46]. To avoid the complexity of Markov models for  $I_{NaL}$  [30], we adapted the Hodgkin-Huxley mathematical description of Hund et al. [46] to human data, as we did in a previous work using ten Tusscher et al. AP model [48]. Our model has been validated against experimental recordings of human APD in normal conditions [8,27,28], APD prolongation under the effect of drugs [29] and voltage clamp experiments [58]. Recently, a new AP model for human ventricular myocytes has been published by O'Hara et al. [43] including a formulation for  $I_{NaL}$  as in the present work. However,

as their AP model includes CaMK regulation, their formulation of  $I_{NaL}$  also includes the effect of CaMK regulation.

In agreement with previous experimental observations, the upregulation of  $I_{NaL}$  during HF [15] or its enhancement in situations of reduced repolarization reserve [21] prolongs APD, causes AP triangulation and increase the reverse rate-dependent prolongation of APD, which are important harbingers for cardiac arrhythmias. The contribution of  $I_{NaL}$  in the reverse APD rate-dependence was also previously simulated by our group [60] using a different human AP model [48] and conditions of reduced repolarization reserve. In regard to HF conditions, there is background of decreased outward currents, and even a small increase of  $I_{NaL}$  becomes more efficient in prolonging APD and favoring the trigger of EADs. The high probability of formation of EAD, leading to TdP, during HF has been experimentally demonstrated [7] but few studies have addressed the role of  $I_{NaL}$  in such situations [18,26,61]. Our results are in keeping with experimental findings, in which the  $I_{NaL}$  blocker ranolazine, effectively suppresses EADs from ventricular myocytes from failing hearts [18,23]. Furthermore, during conditions of reduced repolarization reserve, in which outward  $K^+$  currents are inhibited by drugs or by diseases, results of experimental studies [21–23,62] have unmasked the role of endogenous or enhanced  $I_{NaL}$  in exerting proarrhythmic effects. This is in agreement with the results of our simulations, and provides evidence of this effect in HF. Our findings support the hypothesis that  $I_{NaL}$  represents an important target for triggered-arrhythmias treatment [13]. Similarly, inhibition of CaMKII, which is known to be responsible for  $I_{CaL}$  and ryanodine receptor phosphorylation as well as  $I_{NaL}$  regulation, appears to be an important therapeutic target for suppressing arrhythmias in HF [13,17,63].

### Limitations of the study

Several limitations need to be considered, when drawing conclusions from the present study. The model for  $I_{NaL}$  was formulated on the basis of voltage-clamp human data for mid-myocardial cells [58], as no measurements were available for endocardial or epicardial cells. Indeed,  $I_{NaL}$  is difficult to record due to the very low magnitude ( $\sim 30\text{--}60$  pA) of this current under normal conditions [58]. Maltsev and Undrovinas reported a double exponential decay of  $I_{NaL}$  [58]. However, we chose a single-exponential decay formulation as proposed in previous studies [46,64], and also in the new AP model by O'Hara et al. [43], as this model was able to reproduce its main effects on the AP. To build the model of HF used in the present study, the ionic remodeling was mainly based on experimental data observed in human hearts. Data from a large number of experimental studies have been taken into account, thus resulting in a high variability not only in the ionic remodeling but also in the stage of HF. In addition to the difficulties associated with gaining access to human hearts, explanted diseased hearts are usually in the end stage of HF. Moreover, there are controversies regarding specific ion channel currents remodeling during HF, such as  $I_{NaT}$ ,  $I_{CaL}$ ,  $I_{Kr}$ , and  $I_{Ks}$ , and changes in these currents were not included in our HF model. Their effects on the EP characteristics would be significant contributors to the phenotype. We did however simulate changes in  $I_{NaL}$  concomitant with inhibition of  $I_{Kr}$  and increase in  $I_{CaL}$  (Figures 6 and 8). Finally, our basic HF model has the inherited limitations described for the GPB model. In this sense, improvements related to the rapid phase of APD rate adaptation have been accomplished in later human AP models [34,43]. The response of failing myocytes to abrupt changes of the stimulation rate was however out of the scope of the present study because of the lack of availability of experimental data in HF. With



regard to steady-state APD rate-dependence, the GPB model is accurate except for very low frequencies [43]. Thus the results obtained for APD<sub>90</sub> at low frequencies should be taken with caution.

## Conclusion

This study aimed to investigate in silico the role of I<sub>NaL</sub> in the electrophysiological and Ca<sup>2+</sup> homeostasis phenotype of myocytes from failing hearts. Our results showed that the enhancement of this current during HF can lead to important prolongation of APD and triangulation, increases in reverse rate-dependent prolongation of APD, significantly contributes to Ca<sup>2+</sup> handling changes and has an indirect but pivotal role in the genesis of EADs.

## Methods

### Model of the human ventricular I<sub>NaL</sub>

To simulate the electrical activity of human ventricular myocytes, the AP model formulated by Grandi et al. [25] for endocardial cells was used. This model is one of the latest and most detailed mathematical model for ionic currents and Ca<sup>2+</sup> handling of the human ventricular AP. A particular strength of the GPB model is its ability to reproduce the rate-dependence of APD upon outward K<sup>+</sup> currents block and their individual role in repolarization. Thus, this model provides a powerful tool to explore repolarization abnormalities under conditions of disease, such as HF. However, in order to realistically simulate HF, an important issue remains unresolved in this model, namely the role of I<sub>NaL</sub>.

We included in the GPB model a new formulation for human I<sub>NaL</sub> that is described in equations (1) to (4) and is based on the formulation we included previously [60] in the AP model formulated by ten Tusscher et al. [48].

$$I_{NaL} = g_{NaL} \cdot m_L^3 \cdot h_L \cdot (V - E_{NaL}) \quad (1)$$

$$\frac{dh_L}{dt} = \frac{h_{L,\infty} - h_L}{\tau_{hL}} \quad (2)$$

$$h_{L,\infty} = \frac{1}{1 + e^{((V_m + 91)/6.1)}} \quad (3)$$

$$\tau_{hL} = 233 \text{ ms} \quad (4)$$

We adopted these equations following Hodgkin Huxley formalism, where the activation gate (m<sub>L</sub>) and the Nernst potential for Na<sup>+</sup> (E<sub>NaL</sub>) were unchanged with respect to I<sub>NaT</sub> formulation in the GPB model. The steady-state inactivation gate (h<sub>L,∞</sub>) was taken from Maltsev et al. [64], as did Hund et al. in their model for canine ventricular myocytes [46]. The maximum conductance (g<sub>NaL</sub>) and the time constant of inactivation (τ<sub>hL</sub>) were fitted to reproduce I<sub>NaL</sub> data taken from human mid-myocardial myocytes [58]. In their experiments, Maltsev et al. [58] measured an I<sub>NaL</sub>/I<sub>NaT</sub> ratio of approximately 0.1% using a voltage clamp protocol at room temperature. In our model g<sub>NaL</sub> was fitted accordingly using voltage clamp simulations, yielding 0.015 mS/μF. No correction factor for temperature was applied, as we assumed that I<sub>NaL</sub>/I<sub>NaT</sub> ratio does not change with temperature in myocytes, as reported in transfected HEK-293 cells [65]. However, because experimental

data indicate that ion channel dynamics are altered by temperature, the time constant of inactivation (τ<sub>hL</sub>) of I<sub>NaL</sub> reported by Maltsev et al. [26] for human ventricular mid-myocardial myocytes was multiplied by a Q<sub>10</sub> factor of 2.2 [65], yielding 233 ms at 37°C.

### Heart failure cellular model for human

The remodeling of myocyte electrophysiology in HF is well described [3] and can explain for the most, the hallmark characteristics of failing cardiac tissues and myocytes, such as AP prolongation and alteration of Ca<sup>2+</sup> and Na<sup>+</sup> handling [2,6,66]. On the basis of experimental observations [3,4,7,9,11,13–15,67] and previous simulation studies [5,12,44,47], we hereby propose various changes in the formulation of several ionic currents of the GPB model to reproduce the reported experimental changes in AP and intracellular Ca<sup>2+</sup> and Na<sup>+</sup> handling in ventricular myocytes from failing human hearts [8,31,66]. Our model will be referred to as the basic HF model. Table 1 summarizes the changes we made in the different ionic properties with respect to the GPB model. The ionic remodeling is mainly based on experimental data observed in human hearts.

A prominent increase in I<sub>NaL</sub> and slowing of current decay has been described in ventricular mid-myocardial myocytes isolated from failing hearts of dogs and humans [15,67]. Accordingly, the current density and the time constant of inactivation were increased two-fold compared to non-failing cells. Although Maltsev et al. [15] reported a smaller increase in both values in failing myocytes, their experiments were performed at room temperature and stated that the changes are expected to increase at physiological temperature. Furthermore, the values of the time constant of inactivation could be measured for human myocytes, but the current density was only given for dog myocytes, as the authors state the difficulty to measure it in human myocytes where the variability was very high. Also, in a previous study by Valdivia et al. [16], I<sub>NaL</sub> was increased 5-fold in human failing myocytes with respect to normal myocytes. Downregulation of K<sup>+</sup> currents is the most consistent ionic current change observed in myocytes isolated from failing hearts from animal models and humans [6,7,10]. Mainly, I<sub>to</sub> is downregulated without a significant change in the voltage dependence or kinetics [10]. We reduced the transient outward K<sup>+</sup> current (I<sub>to</sub>) to 40% of its normal value. Reported changes in I<sub>K1</sub> functional expression are more variable than I<sub>to</sub> [3,10] and have a strong dependence on the etiology. The conductance of this ion channel was multiplied by 0.68 as in [12], a value within the experimental range. In regards to [Na<sup>+</sup>]<sub>i</sub> handling, which is also altered in HF, we reduced I<sub>NaK</sub> activity by 10%, as the preponderance of experimental data reveal that the expression and function of the Na<sup>+</sup>/K<sup>+</sup>-ATPase are reduced in HF [3,4,14]. Similar to that of Priebe and Beuckelmann simulation study [5], the background Na<sup>2+</sup> current (I<sub>NaB</sub>) was not included in our HF model. Finally, the changes in intracellular and SR Ca<sup>2+</sup> homeostasis were achieved by an increase of 75% of I<sub>NCX</sub> [5,12,68], and a decrease of 50% of I<sub>SERCA</sub> [11]. To reproduce the experimentally observed changes in Ca<sup>2+</sup> sensitivity of the ryanodine receptor (RyR) [13,69], I<sub>peak</sub> was increased 3-fold and the parameter EC<sub>50SR</sub> for SR [Ca<sup>2+</sup>]-dependent activation of SR release (see Grandi [25] supplementary data) was reduced to 0.4 mM. The background Ca<sup>2+</sup> current was changed as in the study of Priebe and Beuckelmann [5].

### Sensitivity analysis of the HF model

A sensitivity analysis was performed to investigate how the reported variability in HF remodeling data might modulate the main EP characteristics in HF. These characteristics “c” include

**Table 1.** Heart failure remodeling.

	% Change vs. GPB	References	Experimental conditions
$I_{NaL}$	↑ 200	Maltsev et al., 2007 [15]	Isolated cardiomyocytes from LV mid-myocardium of failing dog hearts. Whole cell voltage clamp (room temperature)
		Valdivia et al., 2005 [16]	Isolated cardiomyocytes from LV of failing human hearts. Whole cell voltage clamp (room temperature)
$\delta_{HL}$	↑ 200	Maltsev et al., 2007 [15]	Isolated cardiomyocytes from LV mid-myocardium of failing human hearts. Whole cell voltage clamp (room temperature)
$I_{to}$	↓ 60	Wettwer et al., 1994 [70]	Isolated cardiomyocytes from LV endocardium of failing human hearts. Whole cell voltage clamp (room temperature)
		Beuckelmann et al., 1993 [10]	Isolated cardiomyocytes from LV mid-myocardium of failing human hearts. Whole cell voltage clamp (room temperature)
		Nabauer et al., 1996 [71]	Isolated cardiomyocytes from LV endocardium of failing human hearts. Whole cell voltage clamp (room temperature)
$I_{K1}$	↓ 32	Tomaselli et al., 1999 [3]	Review article. Several species.
		Beuckelmann et al., 1993 [10]	Isolated cardiomyocytes from LV mid-myocardium of failing human hearts. Whole cell voltage clamp (35°C)
		Li et al., 2004 [7]	Isolated cardiomyocytes from RV epicardium of failing human hearts. Whole cell voltage clamp (room temperature)
$I_{NaK}$	↓ 10	Bundgaard et al., 1996 [14]	Measurements of human myocardial. Na,K-ATPase concentration in failing hearts
		Tomaselli et al., 1999 [3]	Review article.
		Tomaselli et al., 2004 [4]	Review article.
$I_{Nab}$	= 0	Priebe and Beuckelmann, 1998 [5]	Simulation of human HF.
$I_{Cab}$	↑ 153	Priebe and Beuckelmann, 1998 [5]	Simulation of human HF.
$NCX$	↑ 175	Priebe and Beuckelmann, 1998 [5]	Simulation of human HF.
		Reinecke et al., 1996 [68]	The functional activity of the Na <sup>+</sup> -Ca <sup>2+</sup> exchanger was determined by measuring the Na <sup>+</sup> -dependent Ca <sup>2+</sup> uptake into membrane vesicles prepared from human left ventricular samples
$SERCA$	↓ 50	Piacentino et al., 2003 [11]	Isolated cardiomyocytes from LV of failing human hearts. Measurements of Ca <sup>2+</sup> uptake rates by the SR (37°C).
		Hasenfuss et al., 1994 [72]	Endocardial strip preparations from human failing hearts. Measurements of Ca <sup>2+</sup> uptake in myocardial homogenates (37°C).
		Schwinger et al., 1995 [73]	LV from human failing hearts. Measurements of Ca <sup>2+</sup> uptake.
$I_{leak}$	↑ 500	Bers et al., 2006 [9]	Review article.
$EC_{50SR}$	↓ 11	Curran et al., 2010 [69]	Isolated cardiomyocytes from LV of failing rabbit hearts. Measurements of RyR sensitivity to SR Ca <sup>2+</sup> .
		Antoons et al., 2007 [13]	Review article.
		Bers et al., 2006 [9]	Review article.

Changes in ion channel, transporters, and pumps activities, and constants used in the basic heart failure (HF) model. The changes are indicated in percentage of increase (↑) or decrease (↓) with respect to the Grandi et al. model (the GPB model) [25].  
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APD at 90% and 50% of repolarization ( $APD_{90}$  and  $APD_{50}$ ), triangulation, peak systolic and diastolic  $[Ca^{2+}]_i$  transient,  $\delta_{Ca}$  decay, peak systolic and diastolic  $[Ca^{2+}]_{SR}$ ,  $[Na^+]_i$ , and  $t_{NCXRP}$ . These characteristics were calculated at steady-state HF conditions (HF basic model) and after varying one parameter “p” at a time. The parameters included each of the ionic current properties modified in the HF basic model, and were varied to its normal value, as in the GPB model, and to a value representing twice that observed in the HF basic remodeling. Although an important change has been implemented (double change) to be considered as a high variability, only the sensitivity, i.e. the relative effect was analyzed. The sensitivity analysis performed similar to that of Romero et al. [32], wherein the indexes percentage of change ( $D_{c,p,x}$ ) and sensitivities ( $S_{c,p}$ ) were calculated as follows:

$$D_{c,p,x} = \frac{C_{p,x} - C_{HF\ basic}}{C_{HF\ basic}} \times 100 \quad (5)$$

$$S_{c,p} = \frac{D_{c,p,2} - D_{c,p,1}}{\Delta_a} \quad (6)$$

with  $C_{p,x}$  and  $C_{HF\ basic}$  being the magnitude of the characteristic c when parameter p undergoes a double change ( $x = 2$ ) with respect to the HF basic change, or no change at all ( $x = 1$ );  $\Delta_a$  is the total interval of change of parameter p.

### Stimulation protocols

Voltage clamp was used to simulate  $I_{NaL}$  behavior. A voltage pulse to  $-20$  mV was applied from a holding potential of  $-120$  mV as depicted in the inset of Figure 1A. To calculate  $I_{NaL}/I_{NaT}$  ratios (see Figure 1C)  $I_{NaL}$  was measured at 40 ms after the application of the depolarizing pulse and was divided by peak  $I_{NaT}$ .

Cellular simulations were conducted at a stimulation rate of 1 Hz. Measurements were taken after achieving steady-state conditions.

Changes in  $[Na^+]_i$  and  $[Ca^{2+}]_i$  levels at various stimulation rates were measured using a staircase protocol as previously described [32,33]. Cardiomyocytes were stimulated at increasing fast frequencies (0.5, 1, 1.5, 2 and 2.5 Hz) for 10 minutes, and  $[Ca^{2+}]_i$  and  $[Na^+]_i$  levels were recorded for each of the frequencies.

To analyze the APD rate-dependence, simulations were performed at different frequencies of stimulation (0.5, 1, 1.25 and 1.6 Hz). Measurements of APD<sub>90</sub> were taken after achieving the steady-state; likewise for APs,  $[Na^+]_i$  and several ionic currents. The rate-dependence was measured as the difference between the maximum APD<sub>90</sub> for a stimulation rate of 0.5 Hz and the minimum APD<sub>90</sub> corresponding to the highest stimulation frequency.

## Supporting Information

**Figure S1 Sensitivity of APD<sub>90</sub> to the  $I_{NaL}$  amplitude in HF.** Steady-state APs at 1 Hz (square symbols and solid lines) and 0.5 Hz (circle symbols and dashed lines) pacing rates with varying  $I_{NaL}/I_{NaT}$  for normal conditions using the GPB model (thick lines) and under basic HF conditions (thin lines) where  $I_{NaL}$  is doubled with respect to normal conditions. The range of experimental APD<sub>90</sub> for human for normal conditions is represented by the two dotted blue lines. The range of experimental APD<sub>90</sub> for human for HF conditions is represented by the two discontinuous red lines. (TIF)

**Figure S2 Sensitivity of APD rate-dependence to variations in individual ionic parameters in HF.** The steady-state APD<sub>90</sub> for different stimulation frequencies is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of one ionic parameter with respect to its value in the basic HF model. (TIF)

**Figure S3 Sensitivity of APD rate-dependence to variations in all ionic parameters in HF.** The steady-state APD<sub>90</sub> for different stimulation frequencies is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of all the ionic parameters simultaneously with respect to their value in the basic HF model. (TIF)

**Figure S4 Sensitivity of rate-dependent changes in systolic  $[Ca^{2+}]_i$  to variations in individual ionic parameters in HF.** Systolic  $[Ca^{2+}]_i$  after 10 minutes of stimulation at increasing rates is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of one ionic parameter with respect to its value in the basic HF model. (TIF)

**Figure S5 Sensitivity of rate-dependent changes in systolic  $[Ca^{2+}]_i$  to variations in all ionic parameters in HF.** Systolic  $[Ca^{2+}]_i$  after 10 minutes of stimulation at increasing rates is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of all the ionic parameters simultaneously with respect to their value in the basic HF model. (TIF)

**Figure S6 Sensitivity of rate-dependent changes in diastolic  $[Ca^{2+}]_i$  to variations in individual ionic parameters**

**in HF.** Diastolic  $[Ca^{2+}]_i$  after 10 minutes of stimulation at increasing rates is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of one ionic parameter with respect to its value in the basic HF model.

(TIF)

**Figure S7 Sensitivity of rate-dependent changes in diastolic  $[Ca^{2+}]_i$  to variations in all ionic parameters in HF.** Diastolic  $[Ca^{2+}]_i$  after 10 minutes of stimulation at increasing rates is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of all the ionic parameters simultaneously with respect to their value in the basic HF model.

(TIF)

**Figure S8 Sensitivity of rate-dependent changes in  $[Na^+]_i$  to variations in individual ionic parameters in HF.**  $[Na^+]_i$  after 10 minutes of stimulation at increasing rates is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of one ionic parameter with respect to its value in the basic HF model.

(TIF)

**Figure S9 Sensitivity of rate-dependent changes in  $[Na^+]_i$  to variations in all ionic parameters in HF.**  $[Na^+]_i$  after 10 minutes of stimulation at increasing rates is shown for normal conditions using the GPB model (thick line), for basic HF conditions (solid line), and for a 15% increase (long dashed line) and a 15% reduction (short dashed line) of all the ionic parameters simultaneously with respect to their value in the basic HF model.

(TIF)

**Figure S10 Sensitivity of EAD generation to variations in individual ionic parameters in HF.** Steady-state APs at 1-Hz pacing rate with 50% inhibition of  $I_{Kr}$ , 30% increase of  $I_{CaL}$ . The simulated results using the basic HF model are shown with a thick line, the solid and dashed lines show the results obtained for a 15% increase and a 15% reduction, respectively, of one ionic parameter with respect to its value in the basic HF model.

(TIF)

**Figure S11 Sensitivity of EAD generation to variations in all ionic parameters in HF.** Steady-state APs at 1-Hz pacing rate with 50% inhibition of  $I_{Kr}$ , 30% increase of  $I_{CaL}$ . The simulated results using the basic HF model are shown with a thick line, the solid and dashed lines show the results obtained for a 15% increase and a 15% reduction, respectively, of all the ionic parameters simultaneously with respect to their value in the basic HF model.

(TIF)

**Figure S12 Mechanisms for early afterdepolarizations with a 50% continuous block of  $I_{rel}$ .** Simulated APs and ionic currents at 1-Hz pacing rate under HF conditions, 50% inhibition of  $I_{Kr}$ , 30% increase of  $I_{CaL}$ . Panel A shows EADs (dark line) with the basic HF value of  $I_{rel}$  and APs with no EADs when  $I_{rel}$  was 50% blocked (light line). The temporal evolutions of  $I_{NaL}$  (panel B),  $[Ca^{2+}]_{SR}$  (panel C),  $I_{rel}$  (panel D), NCX activity (panel E), and activation gate of  $I_{CaL}$  (panel F) are also depicted with the basic HF value of  $I_{rel}$  (dark line) and when  $I_{rel}$  was 50% blocked (light line).

(TIF)

**Figure S13 Mechanisms for early afterdepolarizations with a 50% transitory block of  $I_{rel}$ .** Simulated APs and ionic currents at 1-Hz pacing rate under HF conditions, 50% inhibition of  $I_{Kr}$ , 30% increase of  $I_{CaL}$ . Panel A shows EADs (dark line) with the basic HF value of  $I_{rel}$  and APs with no EADs when  $I_{rel}$  was 50% blocked (light line) during the 5 stimulations indicated by a horizontal line. The temporal evolutions of  $I_{NaL}$  (panel B),  $[Ca^{2+}]_{SR}$  (panel C),  $I_{rel}$  (panel D), NCX activity (panel E), and activation gate of  $I_{CaL}$  (panel F) are also depicted with the basic HF value of  $I_{rel}$  (dark line) and when  $I_{rel}$  was 50% blocked (light line) during the 5 stimulations indicated by a horizontal line. (TIF)

**Figure S14 Mechanisms for early afterdepolarizations with a 50% continuous block of  $I_{NCX}$ .** Simulated APs and ionic currents at 1-Hz pacing rate under HF conditions, 50% inhibition of  $I_{Kr}$ , 30% increase of  $I_{CaL}$ . Panel A shows EADs (dark line) with the basic HF value of  $I_{NCX}$  and APs with no EADs when  $I_{NCX}$  was 50% blocked (light line). The temporal evolutions of  $I_{NaL}$  (panel B),  $[Ca^{2+}]_{SR}$  (panel C),  $I_{rel}$  (panel D), NCX activity (panel E), and activation gate of  $I_{CaL}$  (panel F) are also depicted with the

basic HF value of  $I_{NCX}$  (dark line) and when  $I_{NCX}$  was 50% blocked (light line). (TIF)

**Figure S15 Mechanisms for early afterdepolarizations with a 50% transitory block of  $I_{NCX}$ .** Simulated APs and ionic currents at 1-Hz pacing rate under HF conditions, 50% inhibition of  $I_{Kr}$ , 30% increase of  $I_{CaL}$ . Panel A shows EADs (dark line) with the basic HF value of  $I_{NCX}$  and APs with no EADs when  $I_{NCX}$  was 50% blocked (light line) during the 5 stimulations indicated by a horizontal line. The temporal evolutions of  $I_{NaL}$  (panel B),  $[Ca^{2+}]_{SR}$  (panel C),  $I_{rel}$  (panel D), NCX activity (panel E), and activation gate of  $I_{CaL}$  (panel F) are also depicted with the basic HF value of  $I_{NCX}$  (dark line) and when  $I_{NCX}$  was 50% blocked (light line) during the 5 stimulations indicated by a horizontal line. (TIF)

## Author Contributions

Conceived and designed the experiments: BT. Performed the experiments: KC JFG BT. Analyzed the data: KC JFG BT LB SR JS JMF. Wrote the paper: BT SR LB.

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