Inward Rectifier Current Downregulation Promotes Spontaneous Calcium Release in a Novel Model of Rat Ventricular Electrophysiology

Harley J Stevenson-Cocks, Michael A Colman, Ed White, Alan P Benson

School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom

Abstract

Aberrant intracellular calcium handling, as observed in diseases such as heart failure, promotes lethal ventricular arrhythmias and sudden cardiac death. Recent data from our laboratory suggests that reduced expression of the inward rectifier current in failing rat myocytes increases spontaneous calcium release, however existing computational models are unable to reproduce the underlying stochastic calcium cycling dynamics and so we have been unable to use simulation approaches to explore the cause of this pro-arrhythmic behaviour.

Here, we develop a novel model of rat ventricular electrophysiology that reproduces normal spatio-temporal calcium dynamics. Simulations implementing a similar reduction in inward rectifier current to that observed experimentally show that spontaneous calcium release is promoted by action potential prolongation and sarcoplasmic reticulum loading in the presence of a depolarised resting membrane potential. Combined, these effects can result in triggered activity.

The model therefore provides insight into arrhythmogenic mechanisms in failing ventricular myocytes and can be utilised to further explore pro-arrhythmic behaviour caused by abnormal calcium handling.

1. Introduction

Heart failure (HF) is characterised by an inability of the heart to supply blood to the body, as the ventricles are unable to sufficiently contract or relax in a synchronous manner. Many HF patients die suddenly, largely from ventricular arrhythmias [1] which result in the rapid and terminal reduction of organ perfusion pressure. With the growing prevalance of cardiovascular disease globally, the development of improved preventative and therapeutic strategies is of paramount importance. Thus, greater understanding of the complex underlying processes is necessary to reduce the mortality, morbidity and economic burden of HF.

Aberrant calcium (Ca^{2+}) handling is known to contribute to both the mechanical and electrical dysfunction seen in HF, however dissecting the underlying mechanisms presents a major research challenge as they occur from the meso- to the macroscopic scale and across varying timescales. Accordingly, methodologies beyond traditional experimental ones are increasingly being employed to investigate the pathophysiological processes that result in these disease states. Sophisticated computational models of cardiac electrophysiology have been developed over the past 50+ years that incorporate a myriad of processes, in one to three dimensions, as well as their associated heterogeneities [2].

Yet, despite the common use of the rat as an animal model of cardiovascular disease, existing computational models of Ca^{2+} handling in rat are unable to recreate many experimental observations and so are incapable of offering mechanistic insight. Our laboratory has recently shown that a reduction in inward rectifier channel (I_{K1}) expression promotes spontaneous Ca^{2+} release in failing rat ventricular myocytes [3] (Figure 1), but current rat myocyte computational models do not capture the stochasticity of Ca^{2+} cycling and so it has not been possible to explore this observation further. Furthermore, existing rat electrophysiology models are unable to reproduce action potentials at the fast physiological rates observed experimentally in rat (6 - 8 Hz, e.g. in [4,5]).

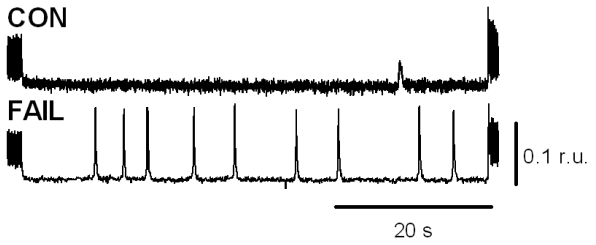

Figure 1. Intracellular Ca^{2+} in control *vs* failing rat ventricular myocytes. Reduced I_{K1} channel expression was found in HF myocytes, which exhibited frequent and substantial spontaneous Ca^{2+} release.

In this study, we develop a novel model of the rat ventricular myocyte which recreates normal electrophysiology and spatio-temporal Ca^{2+} dynamics at physiological

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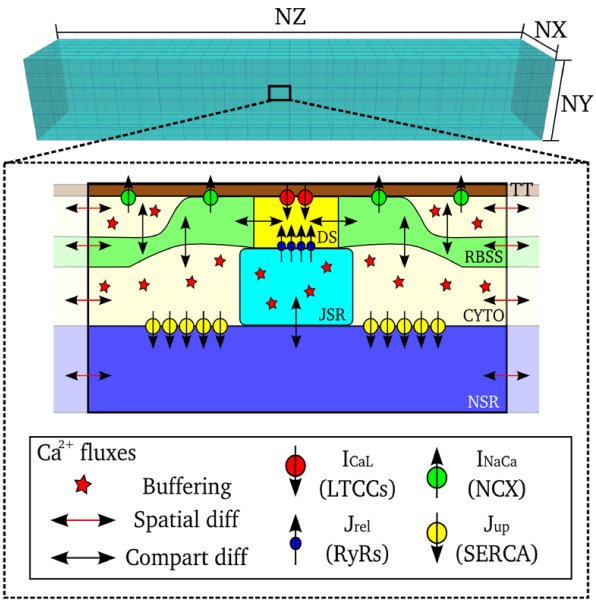

Figure 2. Structure of the spatio-temporal Ca^{2+} handling model. The model accounts for a variable number of calcium release units (CRUs, typically 20,000) each containing multiple compartments (DS, dyadic cleft space into which Ca^{2+}-induced Ca^{2+} release occurs; SS, sub-space; CYTO, bulk cytosolic space; JSR, junctional sarcoplasmic reticulum which interacts with the DS; NSR, network SR). Diffusion occurs between the SS, CYTO and NSR spaces of neighbouring compartments but is restricted within each compartment for the JSR and DS. The membrane currents I_{CaL} and I_{NaCa} are present on the t-tubule (TT) membrane, and the fluxes J_{up} and J_{rel} control Ca^{2+} uptake and release, respectively, from the SR.

heart rates for the rat. We use this newly-developed model to investigate the effects of I_{K1} downregulation on pro-arrhythmic Ca^{2+} handling behaviour in a simulation study.

2. Methods

A novel computational model was constructed by combining a recent model of rat ventricular electrophysiology [6] with a model of stochastic spatio-temporal Ca^{2+} cycling developed in our laboratory [7] (Figure 2), which was then parameterised and validated against experimental data collected at various pacing frequencies. This model served as a control, from which a HF variant was generated by reducing the I_{K1} current conductance parameter, g_{K1}, by 50%, in line with that observed experimentally in our laboratory (mRNA expression of KCNJ2/Kir2.1 reduced by 55% in fail *vs* control myocytes, p=0.01, two-way ANOVA; n=12 [3]). Models were paced to steady-state at a frequency of 8 Hz, followed by a quiescent period during which spontaneous activity could be observed. Changes in electrophysiological or Ca^{2+} handling behaviour were recorded. Models were coded in C/C++ and ran using the University of Leeds ARC3 High Performance Computing facilities.

3. Results

3.1. Model Validation

The developed cell model reproduces whole cell electrophysiology dynamics during control pacing at various cycle lengths and is stable over long simulation durations once steady-state is achieved. Action potential durations (APD, taken as the time to 90% repolarisation) at 1, 6 and 8 Hz were 42.49, 52.29 and 55.83 ms (Figure 3), and these values fell within expected experimental ranges. Representative simulated and experimental values for action potential and Ca^{2+} handling characteristics at 1 Hz are shown in Table 1.

Table 1. Simulated and experimental action potential and Ca^{2+} handling characteristics at 1 Hz.

Parameter	Simulated	Experimental
APD_{90} (ms)	42.49	46.3±8 [8]
Resting potential (mV)	-78.75	-76±2 [8]
AP amplitude (mV)	114.48	108±7 [9]
Systolic Ca^{2+} (uM)	0.29	0.286±0.016 [10]
Ca^{2+} transient duration (ms)	495	486 [11]

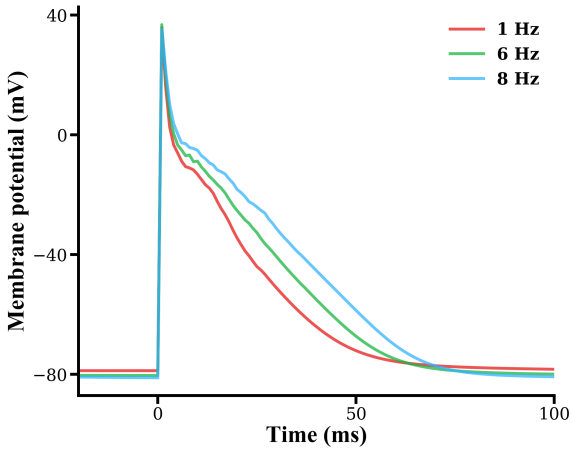

Figure 3. Simulated action potentials. The model reproduced action potential characteristics as observed experimentally at 1, 6 and 8 Hz. Average of 10 simulations shown per pacing frequency.

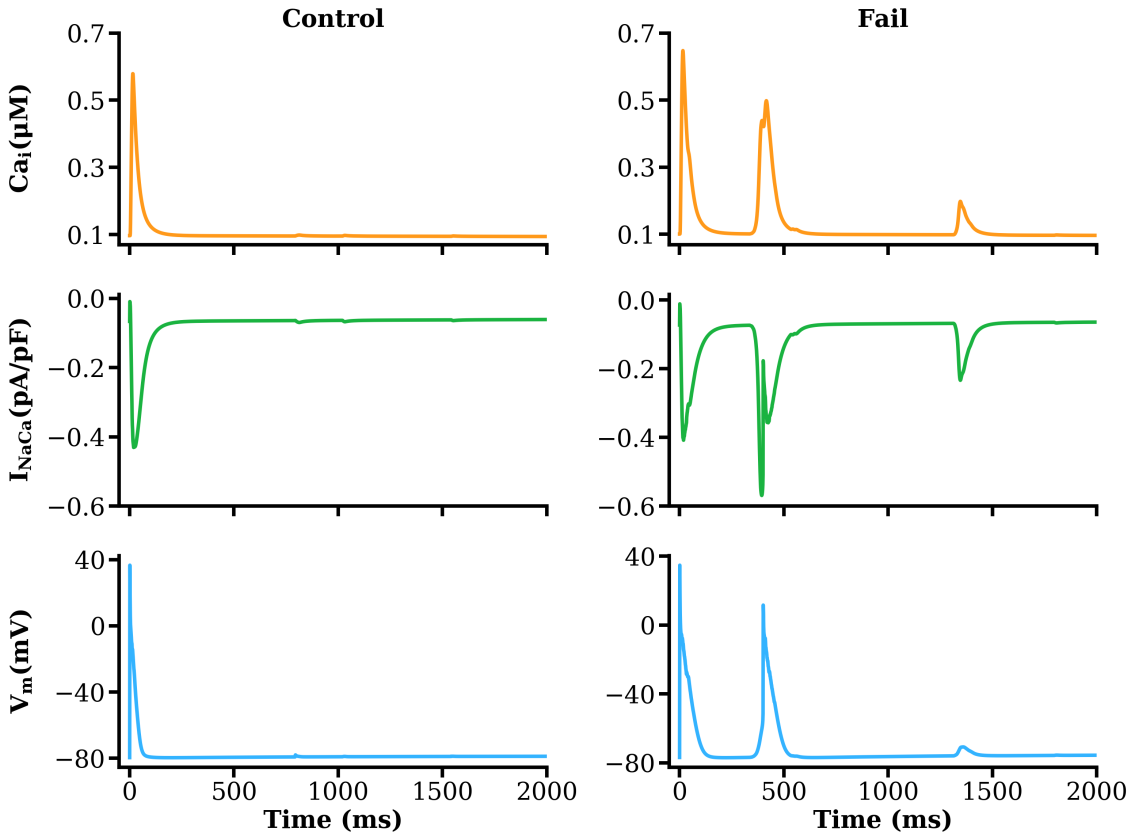

Figure 4. Effects of I_{K1} downregulation on spontaneous activity. Left: Control simulations. No spontaneous activity occurs after steady-state pacing and so there is no diastolic depolarisation or erroneous I_{NaCa} activation. Right: Fail simulations. A large spontaneous Ca^{2+} release is seen, leading to inward I_{NaCa} and triggered activity at 400 ms. Resting membrane potential is also depolarised.

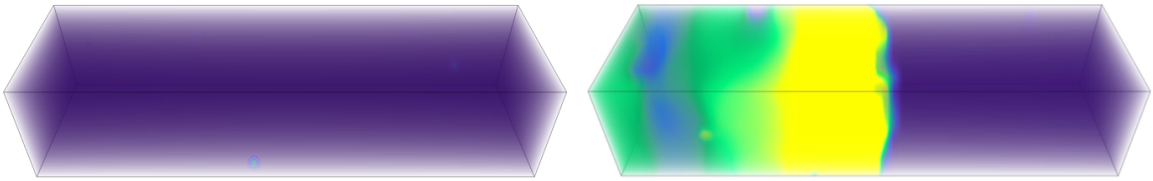

Figure 5. Ca^{2+} waves across the CRUs at t = 500 ms. Left: Control. No spontaneous Ca^{2+} wave is observed. Right: Fail. Spontaneous Ca^{2+} release from CRUs manifests as a propagating Ca^{2+} wave, resulting in triggered activity.

3.2. Inward rectifier current downregulation promotes spontaneous Ca^{2+} release

Implementing a 50% reduction in g_{K1}, the I_{K1} conductance parameter, resulted in a 57% prolongation of steady-state APD *vs* control, from 58.1 to 91.4 ms. In addition, resting membrane potential in HF simulations was depolarised by 3.3 mV, from -78.8 to -75.5 mV.

Spontaneous activity is illustrated in Figure 4. In control simulations (left), there is no spontaneous Ca^{2+} release during the quiescent period after steady-state pacing (top), which is reflected by no erroneous activation of the sodium-calcium exchanger (I_{NaCa}, middle) and no subsequent impact on membrane potential (V_m, bottom). Conversely, in HF simulations, a large spontaneous Ca^{2+} release (peak $[Ca^{2+}]_i$ = 0.498 uM) activates forward-mode

(depolarising) I_{NaCa} and results in a triggered action potential at 400 ms. A second, smaller spontaneous release (peak $[Ca^{2+}]_i$ = 0.198 uM) is observed later leading to a sub-threshold depolarisation of membrane potential by 8.19 mV to -70.82 mV. The manifestation of the larger release at 400 ms as a propagating Ca^{2+} wave is shown in Figure 5, as is its absence in the control simulation.

4. Discussion and Conclusions

We have developed a novel model of rat ventricular myocyte electrophysiology which has reproduced experimental variability in APD from other laboratories. The new model accounts for stochastic spatio-temporal Ca^{2+} handling dynamics alongside membrane ion channel electrophysiology and has provided insight into the mechanisms underlying increased spontaneous Ca^{2+} release in HF resulting from a reduced I_{K1} current; that the resultant prolongation in APD allows more time for loading of the SR with Ca^{2+}, promoting spontaneous Ca^{2+} release events. These releases result in forward-mode, depolarising sodium-calcium exchanger (I_{NaCa}) activity which in turn cause triggered action potentials. Combined with a destabilised membrane (resting membrane potential was depolarised in the HF simulation), this provides a trigger for arrhythmia development in failing myocytes.

Thus, the model provides a supplementary and standalone research tool which can be used to explore how sub-cellular changes associated with HF influence pro-arrhythmic activity at the single cell level. Incorporation into tissue-level simulation protocols may reveal the role of such remodelling in the development of ectopic activity and generation of arrhythmias at the organ level.

Acknowledgements

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Address for correspondence:

Harley J. Stevenson-Cocks

School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK

bs12hjsc@leeds.ac.uk