



UNIVERSITY OF LEEDS

This is a repository copy of *Inward Rectifier Current Downregulation Promotes Spontaneous Calcium Release in a Novel Model of Rat Ventricular Electrophysiology*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/152234/>

Version: Accepted Version

Proceedings Paper:

Stevenson-Cocks, HJ orcid.org/0000-0003-1044-3545, Colman, MA orcid.org/0000-0003-2817-8508, White, E orcid.org/0000-0002-3954-9792 et al. (1 more author) (2019) Inward Rectifier Current Downregulation Promotes Spontaneous Calcium Release in a Novel Model of Rat Ventricular Electrophysiology. In: Computing in Cardiology. 2018 Computing in Cardiology Conference (CinC), 23-26 Sep 2018, Maastricht, Netherlands. IEEE . ISBN 9781728109589

10.22489/CinC.2018.156

© 2018, IEEE. Personal use of this material is permitted. Permission from IEEE must be obtained for all other uses, in any current or future media, including reprinting/republishing this material for advertising or promotional purposes, creating new collective works, for resale or redistribution to servers or lists, or reuse of any copyrighted component of this work in other works.

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

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 prevalence 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 time-scales. 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 ($I_{\text{K}1}$) 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]).

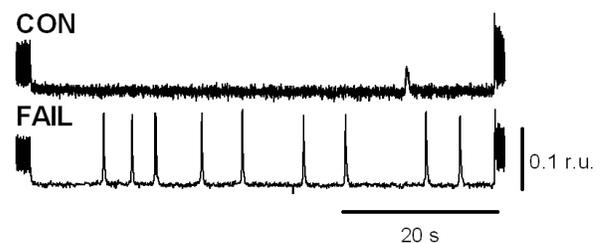


Figure 1. Intracellular Ca^{2+} in control vs failing rat ventricular myocytes. Reduced $I_{\text{K}1}$ channel expression was found in HF myocytes, which exhibited frequent and substantial spontaneous Ca^{2+} release.

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

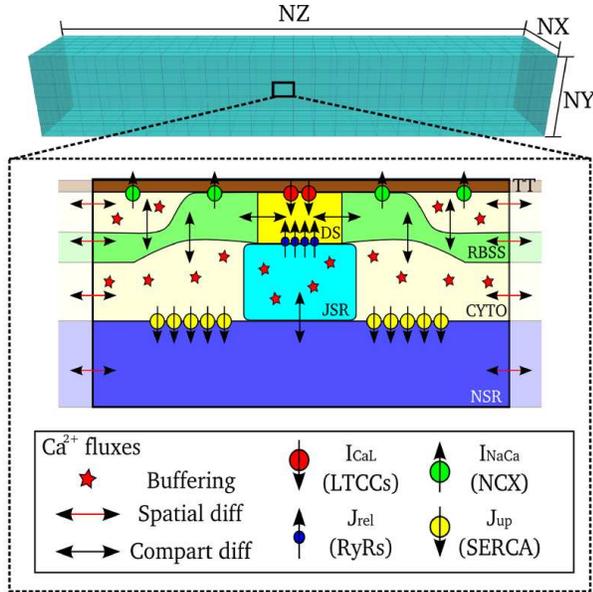


Figure 2. Structure of the spatio-temporal Ca^{2+} handling model. The model accounts for a variable number of calcium release units (CRUs, typically 20,000) each containing multiple compartments (DS, dyadic cleft space into which Ca^{2+} -induced Ca^{2+} release occurs; SS, sub-space; CYTO, bulk cytosolic 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 proarrhythmic 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]

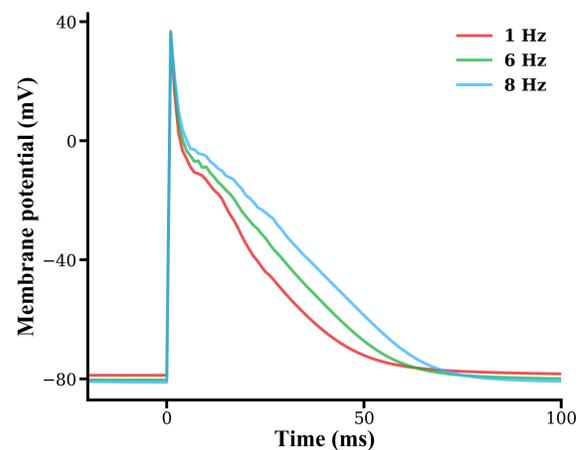


Figure 3. Simulated action potentials. The model reproduced action potential characteristics as observed experimentally at 1, 6 and 8 Hz. Average of 10 simulations shown per pacing frequency.

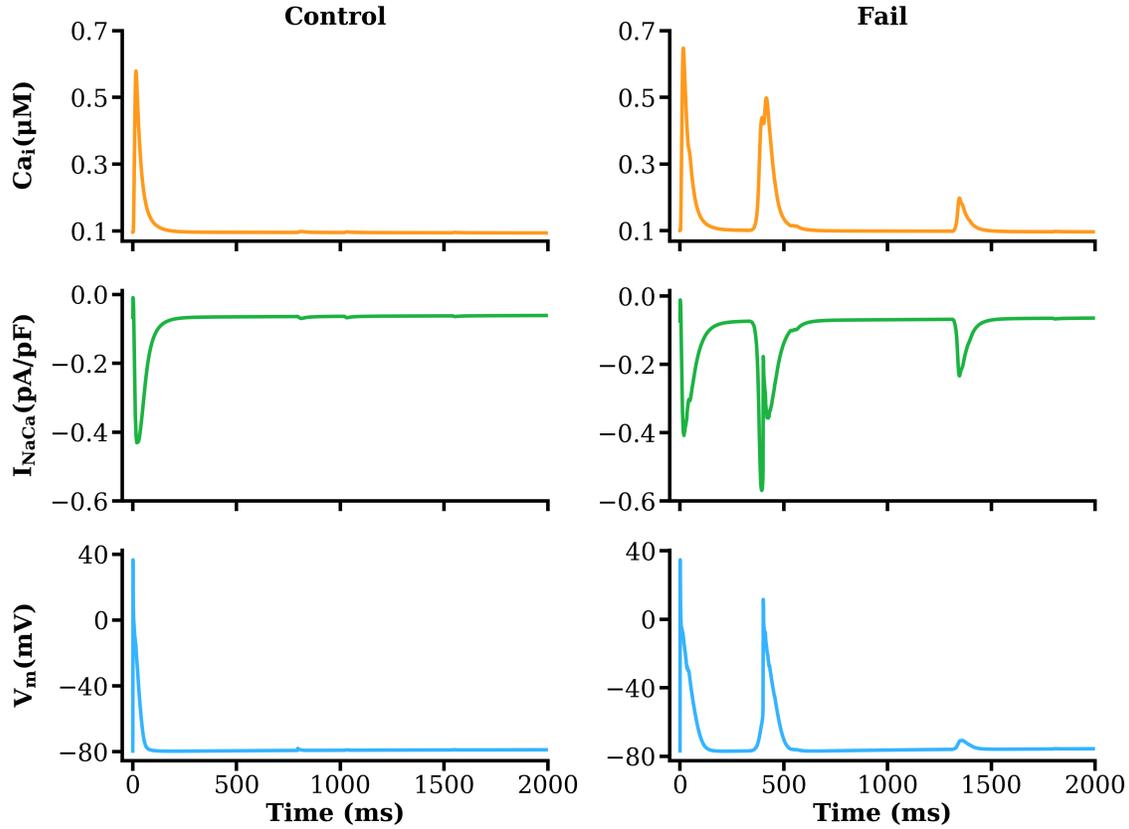


Figure 4. Effects of I_{K1} downregulation on spontaneous activity. Left: Control simulations. No spontaneous activity occurs after steady-state pacing and so there is no diastolic depolarisation or erroneous I_{NaCa} activation. Right: Fail simulations. A large spontaneous Ca^{2+} release is seen, leading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

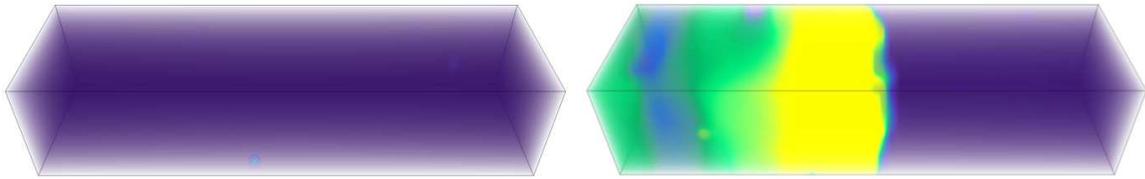


Figure 5. Ca^{2+} waves across the CRUs at $t = 500$ ms. Left: Control. No spontaneous Ca^{2+} wave is observed. Right: Fail. Spontaneous Ca^{2+} release from CRUs manifests as a propagating Ca^{2+} wave, resulting in triggered activity.

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 depo-

larised 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 $[Ca^{2+}]_i = 0.498$ μM) activates forward-mode

(depolarising) I_{NaCa} and results in a triggered action potential at 400 ms. A second, smaller spontaneous release (peak $[Ca^{2+}]_i = 0.198 \mu M$) 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 stand-alone 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

This research was funded by a University of Leeds PhD studentship, a Medical Research Council Strategic Skills Fellowship (MR/M014967/1) and a British Heart Foundation project grant (PG/16/74/32374).

References

- [1] Saxon LA, Bristow MR, Boehmer J, Krueger S, Kass DA, De Marco T, Carson P, DiCarlo L, Feldman AM, Galle E, Ecklund F. Predictors of sudden cardiac death and appropriate shock in the comparison of medical therapy, pacing, and defibrillation in heart failure (companion) trial. *Circulation* 2006;114:2766–2772.
- [2] Roberts BN, Yang PC, Behrens SB, Moreno JD, Clancy CE. Computational approaches to understand cardiac electrophysiology and arrhythmias. *American Journal of Physiology Heart and Circulatory Physiology* 2012;303(7):H766–H783.
- [3] Benoist D, Stones R, Drinkhill M, Bernus O, White E. Arrhythmogenic substrate in hearts of rats with monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy. *American Journal of Physiology Heart and Circulatory Physiology* 2011;300:H2230–H2237.
- [4] Carnevali L, Sgoifo A. Vagal modulation of resting heart rate in rats: the role of stress, psychosocial factors, and physical exercise. *Frontiers in Physiology* 2014;5.
- [5] Sadredini M, Danielsen TK, Aronsen JM, Manothepan R, Hougen K, Sjaastad I, Stokke MK. Beta-adrenoceptor stimulation reveals Ca^{2+} waves and sarcoplasmic reticulum Ca^{2+} depletion in left ventricular cardiomyocytes from post-infarction rats with and without heart failure. *Plos One* 2016;11.
- [6] Gattoni S, Roe AT, Frisk M, Louch WE, Niederer SA, Smith NP. The calcium-frequency response in the rat ventricular myocyte: an experimental and modelling study. *Journal of Physiology London* 2016;594:4193–4224.
- [7] Colman MA, Pinali C, Trafford AW, Zhang HG, Kitmitto A. A computational model of spatio-temporal cardiac intracellular calcium handling with realistic structure and spatial flux distribution from sarcoplasmic reticulum and t-tubule reconstructions. *Plos Computational Biology* 2017;13.
- [8] Perez-Cortes EJ, Islas AA, Arevalo JP, Mancilla C, Monjaraz E, Salinas-Stefanon EM. Modulation of the transient outward current (I_{to}) in rat cardiac myocytes and human $K(v)4.3$ channels by mefloquine. *Toxicology and Applied Pharmacology* 2015;288:203–212.
- [9] Ali RM, Al Kury LT, Yang KHS, Qureshi A, Rajesh M, Galadari S, Shuba YM, Howarth FC, Oz M. Effects of cannabidiol on contractions and calcium signaling in rat ventricular myocytes. *Cell Calcium* 2015;57:290–299.
- [10] Lagadic-Gossmann D, Buckler KJ, LePrigent K, Feuvray D. Altered Ca^{2+} handling in ventricular myocytes isolated from diabetic rats. *American Journal of Physiology Heart and Circulatory Physiology* 1996;270:H1529–H1537.
- [11] Olson ML, Kargacin ME, Ward CA, Kargacin GJ. Effects of phloretin and phloridzin on Ca^{2+} handling, the action potential, and ion currents in rat ventricular myocytes. *Journal of Pharmacology and Experimental Therapeutics* 2007;321:921–929.

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