Loss of Transverse-Tubules Promotes the Development of Ectopic Activity in Guinea-pig Ventricle

Michael A Colman[1], Sanjay Kharche[1,2], Henggui Zhang[1]

[1]Univesity of Manchester, Manchester, UK
[2]University of Exeter, Exeter, UK

Abstract

Intracellular calcium cycling plays an important role in healthy and pathophysiological cardiac behavior. One such behavior is the development of ectopic activity, which is linked to rapid arrhythmias such as tachycardia and fibrillation. Calcium dynamics are strongly dependant on the function of the dyad; a restricted space which contains the L-type calcium channels and intracellular calcium release units. Dyads are observed in both the surface and T-tubular membrane.

In this study, we use a stochastic mathematical model of the guinea-pig ventricle to investigate the effect of a loss of the T-tubular membrane on the dynamics of intracellular calcium cycling and its affect on membrane ion currents. It was demonstrated that in the presence of beta-stimulation, loss of T-tubules promotes significant triggered activity at the cellular level, which may develop into ectopic activity at the tissue level. The primary mechanism by which this occurs is two-fold: (1) increased activity of L-type calcium channels under beta-stimulation leads to large triggered and spontaneous intracellular calcium transients, promoting enhanced sodium-calcium exchanger activity; (2) a loss of the time-independent potassium channels raises the resting potential, allowing the threshold for activation of the fast sodium current to be surpassed.

1. Introduction

Intracellular calcium cycling plays an important role in cardiac single cellular function [1]. In the healthy heart, calcium cycling regulates cellular contraction and calcium oscillations have been implicated in the periodic and spontaneous activity of the cardiac pacemakers. The dynamics of membrane ion transporters and intracellular calcium cycling are closely coupled, and calcium dysfunction has been implicated in arrhythmogenesis through its affects on membrane systems [1].

The calcium-induced-calcium-release (CICR) cycle involves calcium entering the cell through the L-type calcium channels (associated with the L-type calcium current, I_{CaL}), which open during the action potential (AP). These channels are located along the cell membrane in dyads; restricted spaces which contain L-type calcium channels and ryanodine receptors (RyR). The RyR are calcium sensitive structures which lie in the membrane of the sarcoplasmic reticulum (SR), an intracellular calcium store. When calcium enters the cell through the L-type calcium channels, it rapidly binds with the RyR in the dyad space and causes release of calcium from the SR into the cytosolic space. Upon cellular relaxation, calcium concentration in the SR is restored through the SR uptake units, and is extruded from the cell through the sodium-calcium exchanger current (I_{NaCa}). At membrane potentials corresponding to the resting potential, I_{NaCa} is an inward current and can therefore cause a membrane depolarization. Such a mechanism has been implicated in the generation of delayed after-depolarizations (DADs) and triggered activity (TA), resulting from I_{NaCa} activation following spontaneous calcium sparks (RyR calcium release not mediated by the activation of L-type calcium channels during an AP).

DADs leading to TA can result in ectopic activity; that is, irregular spontaneous beats originating from regions of

Table 1. Fraction of ion transporters in the T-tubules (TTs) [2]

Current/transporter	Fraction in TTs
I_{Na}	0.57
I_{CaL}	0.64
I_{NaCa}	0.64
I_{K1}	0.8
I_{Kr}	0.526
I_{Ks}	0.526
I_{Kp}	0.526
I_{Nab}	0.526
I_{NaK}	0.526
Other dyad currents	0.64

the heart other than the cardiac pacemakers. Ectopic activity is linked with rapid arrhythmias in both the ventricles and atria and has been implicated in the generation of re-entry. Dysfunction of the calcium system may promote DADs and TA and therefore mediate the development of tachycardia and fibrillation. Hence, understanding of the mechanisms underlying such activity is imperative in the understanding and treatment of many life-threatening disorders.

T-tubules are invaginations in the cellular membrane which conduct the action potential into the interior of the cell. They may play an important role in governing coordinated cellular contraction by bringing calcium into the interior of the cell, and contain 60-80% of the L-type calcium channels [4] (and hence dyads). Loss of T-tubules (detubulation) is observed in heart conditions which are also associated with ectopic activity. The aim of this study is to investigate the role of detubulation on the behavior of calcium sparks and arrhythmogenesis.

2. Methods

To investigate the role of detubulation on calcium sparks and TA, a multi-scale model of the guinea-pig ventricular myocyte [3] is used and updated, accounting for stochastic behavior in the dyad and coupled surface and T-tubular membrane systems.

2.1. Dyad and T-tubule model

The Gaur-Rudy model of the guinea-pig ventricular AP accounts for stochastic behavior in the dyad [3]. 100 dyads are modeled, each containing 15 L-type calcium channels and 200 RyRs, as well as other currents related to calcium cycling (such as I_{NaCa} and the sarcolemmal calcium pump). The dynamics of I_{CaL} and RyR are each modeled by Markov chains with state transitions given stochastically by the generation of random numbers.

For the present study, the Gaur-Rudy model is updated to account for the heterogeneous coupled surface and T-tubule membrane system, based on the model of Pasek *et al.*[2]. The fraction of membrane currents is not equal in each system (Table 1) and nor is the total surface area of each membrane. As such, the current densities are adjusted for the surface and T-tubular AP. Because more I_{CaL} is observed in the T-tubules, the resulting AP at the surface membrane is shorter than that of the T-tubular membrane [2]. It has been shown that dyads in the surface membrane are no different to those along the T-tubules [4]. Hence, where data are lacking, currents found in the dyads are assumed to have the same distribution between the surface and T-tubule membrane as $I_{CaL,}$ assuming that dyads are distributed according to I_{CaL} distribution. In the rat ventricle, the distribution of I_{NaCa} between each membrane is different to that of I_{CaL}, suggesting this assumption may not hold. However, no such data are available in guinea-pig, and the significant differences between these two species (e.g. the T-tubule network accounts for 52.6% [2] of the total sarcolemmal area in guinea pig, compared to ~30% in rat [4]) mean that such data are not accounted for in the model.

The total number of dyads modeled is increased from 100 to 200 such that 100 dyads may be associated with each system. The current densities are not scaled within each dyad but instead the contribution of the 100 dyads to whole-cell currents and concentrations is scaled according to the fraction of I_{CaL} in each system. The dyads of each system are coupled, permitting calcium diffusion from the surface through the T-tubule. Furthermore, the action potential associated with each membrane is also coupled, as in [2].

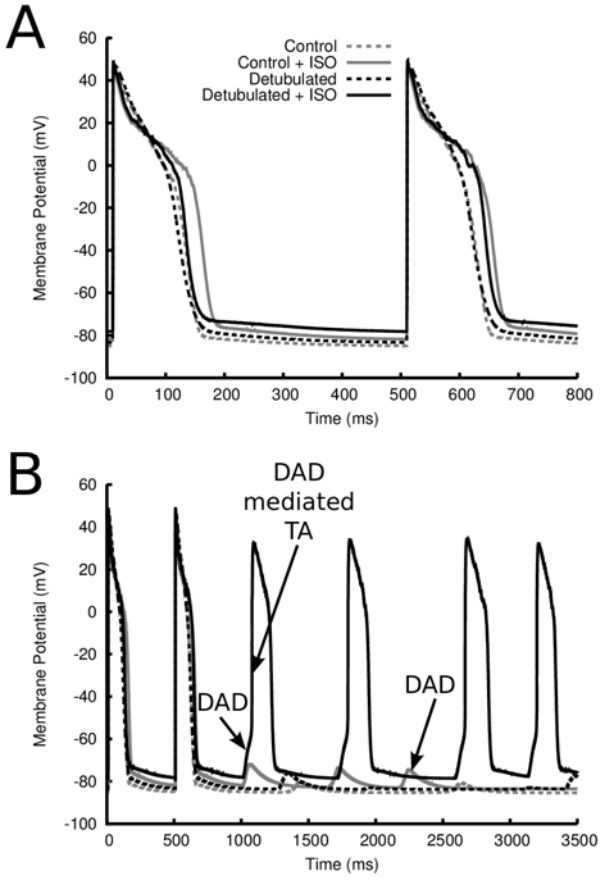

Figure 1. Effects of detubulation and ISO on AP morphology (A) and triggered activity (B). Control refers to full T-tubule network whereas detubulation is a complete loss of T-tubules. Action potentials in (A) are the final two elicited by a train of 100 stimuli and correspond to the first two shown in (B).

2.2. Simulating β-stimulation and loss of T-tubules

β-adrenergic stimulation in the presence of isoprenaline (ISO) is simulated according to Faber *et al.*[5]. Detubulation is simulated by (a) a loss of the membrane currents associated with the T-tubules [6] and (b) decoupling of the T-tubule and surface membrane AP [2]. Dyads associated with the T-tubules are still modeled, but include only RyRs and no membrane transporters.

2.3. Simulation protocols

The model is paced for 100 beats at fast (cycle length = 500 ms) and slow (cycle length = 1500 ms) rates, and then left to run for a further ten seconds. This is repeated for different conditions to determine the role of detubulation and ISO in the development of TA.

3. Results

3.1. Loss of T-tubules shortens the AP

A slight shortening of the AP is observed following loss of the T-tubules (Fig. 1A), primarily due to a lower density of I_{CaL} in the surface membrane compared to the T-tubule network [2,4]. Such an effect is also observed in the presence of ISO, which prolongs the AP compared to control conditions (Fig. 1A). Furthermore, both ISO and detubulation result in a positive shift of the resting potential by 2-4 mV (Fig. 1A) due to the difference in I_{K1} density between the surface and T-tubule network (Table 1) and the effects of ISO on I_{K1} morphology [5]. Note that due to the stochastic nature of the simulations, AP duration varies from beat to beat.

3.2. Detubulation promotes triggered activity

Loss of T-tubules promotes DAD-mediated TA (Fig. 1B). In all simulations, DADs are observed following a train of 100 applied stimuli at fast and slow pacing rates (e.g. Fig. 1B). The magnitude of DADs is increased significantly in the presence of ISO. However, only in the case of detubulation in the presence of ISO did DADs develop into full TA, initiating single or multiple spontaneous APs. In ~50% of simulations under these conditions, multiple DAD-mediated triggered APs were observed, continuing for the duration of the simulation (ten seconds following the final applied stimulus). In ~40% of simulations a single DAD-mediated spontaneous AP was observed, followed by lower amplitude DADs which did not develop into TA. In ~10% of simulations, DADs did not develop into TA.

TA in these cases relies on the activity of I_{NaCa} following spontaneous calcium sparks (Fig. 2). Loss of T-tubules results in a reduction of the overall density of I_{NaCa}, but has no effect on I_{NaCa} density in the surface membrane. Hence, loss of whole-cell I_{NaCa} does not inhibit the ability of I_{NaCa} to drive DADs in the surface membrane.

Furthermore, the elicitation of a spontaneous AP promotes the development of further DADs and TA. The frequency and magnitude of spontaneous calcium sparks depends on calcium entry into the cell; larger amplitude spontaneous calcium transients elicit larger amplitude I_{NaCa} and occur following larger triggered calcium transients. Hence, where a DAD leads to a spontaneous AP, I_{CaL} is reactivated and therefore brings more calcium into the cell. This can then result in subsequent high-amplitude calcium sparks which may elicit further APs, and the cycle repeats (Fig. 2D). Where DADs do not elicit spontaneous APs, the average magnitude of

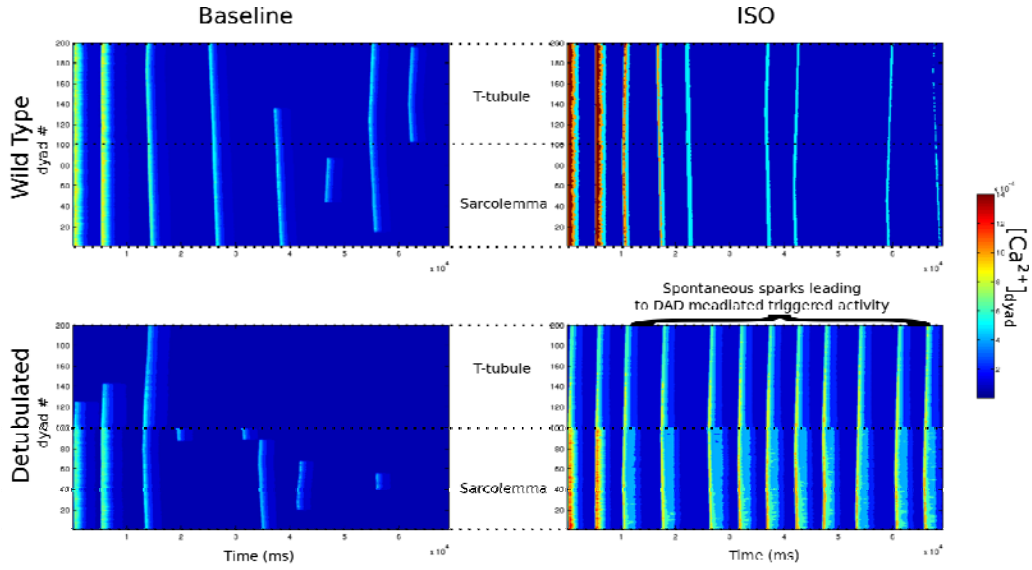

Figure 2. Calcium sparks in model dyads in control (wild type, full T-tubules) and detubulated conditions in the presence and absence of ISO. Dyads associated with each membrane are labelled. The first two sparks are triggered whereas all others are spontaneous.

spontaneous calcium sparks and elicited DADs decreases over time (Fig. 2).

3.3. I_{CaL} and I_{K1} play critical roles in triggered activity

Both I_{CaL} and I_{K1} play critical roles in the mediation of TA (Fig. 3). The increased activity of I_{CaL} in the presence of ISO increases the magnitude of the calcium transient. Such an effect promotes spontaneous calcium sparks of higher magnitude (Fig. 2B,D) which result in high activity of I_{NaCa} (Fig. 3B). However, this alone is insufficient to result in TA; note that the magnitude of I_{NaCa} following a spontaneous calcium spark is similar in cases where I_{K1} is either not affected by ISO or assumed to have equal density in the surface and T-tubule membrane (Fig. 3B, arrows), and yet does not result in TA. Hence, I_{K1} also plays a critical role.

The primary mechanism by which detubulation and ISO effects on I_{K1} promote TA is through the positive shift of the resting potential (Fig. 1A, section 3.1). Such an effect means that (a) I_{NaCa} of the same magnitude results in a DAD of greater amplitude and (b) this DAD crosses the threshold for I_{Na} activation, therefore resulting in TA (Fig. 3).

4. Conclusion

Using a computational model of the Guinea-pig ventricle it has been demonstrated that loss of T-tubules promotes the development of DAD-mediated TA in the presence of ISO. This occurs through the combined effect of an increase in I_{CaL} activity and a positive shift of the resting potential, allowing DADs to surpass the excitation threshold.

Acknowledgements

M.A.C is funded by an EPSRC Doctoral Prize. S.K is supported by EPSRC grants (EP/I029664/1, EP/I029826/1, EP/I030158/1).

Address for correspondence:

Michael A. Colman
Room 3.01
3rd Floor, Schuster Laboratory
Biological Physics Group
Dept. of Physics and Astronomy
University of Manchester
Oxford Road, Manchester, UK
M13 9PL
michael.colman@manchester.ac.uk

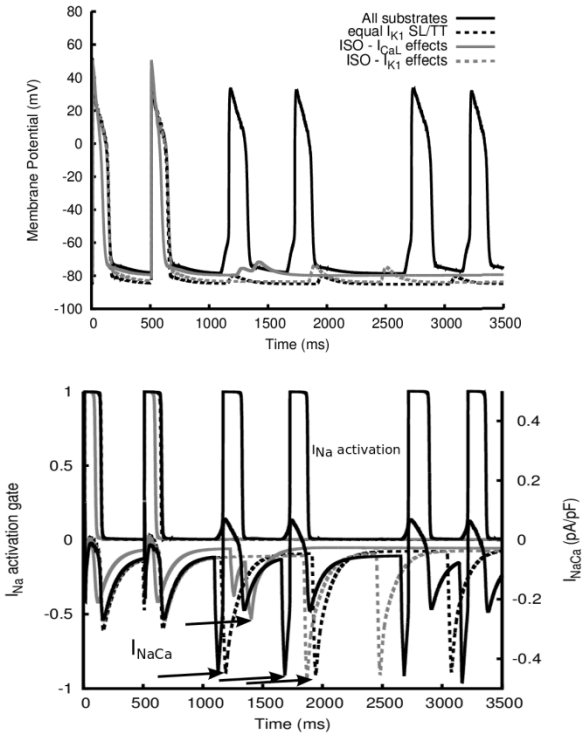

Figure 3. Mechanisms of triggered activity. TA is observed only when ISO effects on I_{CaL} and I_{K1} and the heterogeneous distribution of I_{K1} between membranes is considered. SL = surface sarcolemma, TT = T-tubules.

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