Stability of Genetically Engineered Cardiac Pacemaker - Role of Intracellular Ca^{2+} Handling

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Abstract

Down-regulation of Kir2.1 channel reduces the inward-rectifier potassium current (i_{K1}), and transforms excitable ventricular myocytes to pacemaker cells. This provides a possible bio-technique to induce a biological pacemaker, as an alternative to an implantable electronic pacemaker. However there are two fundamental issues: (i) the stability of the pacemaker activity; (ii) the critical size of the biological pacemaker necessary for robust pacing and driving the surrounding ventricular muscle. In this study, we address the two issues by computer modelling.

1. Introduction

Implantation of electronic pacemakers is widely used to treat patients with diseased cardiac pacemaking or conduction systems, such as the sick sinus syndrome (by an atrial pacing) or atrioventricular node conduction block (by bi-ventricular pacing). However, implementation of electronic pacemakers is not an optimal therapy because of limited battery life time, surgical problems in implantation and attachment of electrodes in cardiac tissue, and the lack of direct response to autonomic nervous system control. Recently strategies using gene therapy have been proposed to develop biological pacemaker as an alternative [1, 2].

In their pioneering work, Miake et al. [1] has shown that downregulating expression of the Kir2.1 channel, which is responsible for the inward rectified potassium current i_{K1} can turn a normal ventricular cell into a pacemaking cell producing spontaneous and rhythmic action potentials (APs). The generated APs have characteristics similar to those of natural pacemaking cells, the sinoatrial node (SA node) and can be modulated by application of isoprenaline, a mimica the action of the sympathetic nervous system [1]. Thus, such a genetically engineered biological pacemaker has been proposed to supplement/replace the use of electronic pacemaker in the future. However, several fundamental questions still remain unclear, including (i) What are the ionic mechanisms underlying the genesis of pacemaker activity in a ventricular myocyte produced by depressing i_{K1}, as it lacks the major pacemaking currents, such as i_{Na}, i_f, $i_{Ca,T}$ present in a SA node cell? (ii) Is the pacemaker activity stable? It is well known that during a sequence of high rate excitations, the homeostasis of Ca^{2+} handling in a ventricular myocyte is disturbed [3] as the Ca^{2+} extrusion flux by Na^+/Ca^{2+} exchanger cannot balance the Ca^{2+} influx by i_{CaL} during each action potential over a long (over 10s time) course, as the former is slower. This leads to accumulation of intracellular Ca^{2+} concentration, in which both diastolic levels and systolic peaks of the intracellular Ca^{2+} transient drift up with time [3]. It is unclear how this unstable Ca^{2+} ion cycling would affect the stability of a biological pacemaker. (iii) What is the critical size of the bio-engineered pacemaking tissue required to overcome the depression from the surrounding ventricular tissue due to electrotonic coupling between them? In this study, we address these questions by computational approaches.

2. Methods

Single cell modelling. A single cell model of electrical activity of human ventricle [4] – the PB model - was used to investigate the ionic mechanisms underlying the pacemaker activity of ventricular myocytes when i_{K1} is depressed. APs, individual ionic channel currents, total net ionic current and intracellular Ca^{2+} concentration during APs were computed over 10 s to explore the ionic mechanisms underlying the genesis of spontaneous and rhythmic APs and the transient nature of the spontaneous activity. Role of $[Ca^{2+}]_i$ on pacemaking stability was investigated by buffering $[Ca^{2+}]_i$ to a constant level of 250 nM.

Multi-cellular tissue model. Initiation and conduction of pacemaker activity was simulated in a multi-cellular tissue model of a ventricular strand, which was developed by incorporating the single cell PB model into one-dimensional (1D) partial differential equation (PDE), which takes the form:

$$\frac{dV(X,t)}{dt} = -\frac{1}{C_m}\sum_j i_j(X,V,t) + \nabla(D(X)\nabla V(X,t)) \quad (1).$$

V is the membrane potential, i_j the j-th gated membrane ionic channel current, C_m the cell membrane capacitance. X defines the physical space of the ventricle tissue. Details of equations and parameters describing the kinetics of membrane ion channels and changes of extracelluar and intracellular ion concentrations can be found in [4]. D is the diffusion coefficient that simulates the intercellular (gap junctional) electrical coupling between ventricular myocytes. In simulations, D was set to 0.00045 cm^2 ms^{-1} that gives a conduction velocity of a planar wave of 0.4 m s^{-1} in the tissue.

In the model, the strand was divided into three segments. In the centre of the strand, there was an auto-rhythmic region (with a variable length), which was coupled to a normal ventricular strand at each side (within a total length of 32 mm). Numerically the 1D PDE model was solved by the explicit Euler method with a space step of 0.1 mm and time step of 0.01 ms, both of which are sufficiently small for stable numerical solutions. The program was coded in C^{++} and run on a SunBlade 2000 Solaris 5.8 Unix system.

3. Results

Figure 1A shows the simulated action potentials from the PB membrane model under normal (control) and i_{K1} reduction (80% block i_{K1}) conditions, generated by a supra-threshold stimulus (with an amplitude of -20 $\mu A/cm^2$ and a duration of 2 ms). Time courses of i_{K1} (Figure 1B) and total net current (Figure 1C) during AP are also shown. Under the control condition, in the late phase of AP repolarization, there was a large outward i_{K1}, which repolarised cell membrane potential to be around -81 mV. Around this potential, i_{K1}, together with other outward currents, balanced inward current resulting in a zero total net current, which facilitated a stable resting potential. With a 80% reduction, the residual i_{K1} was small and there was insufficient outward current. As a result, the maximal diastolic potential (MDP) of AP was elevated to more positive value. The new MDP is unstable as there was a non-zero inward total net current, which depolarised cell membrane potential, but was not large enough to produce an upstroke and then a full AP. Eventually the cell model reached a stable equilibrium potential.

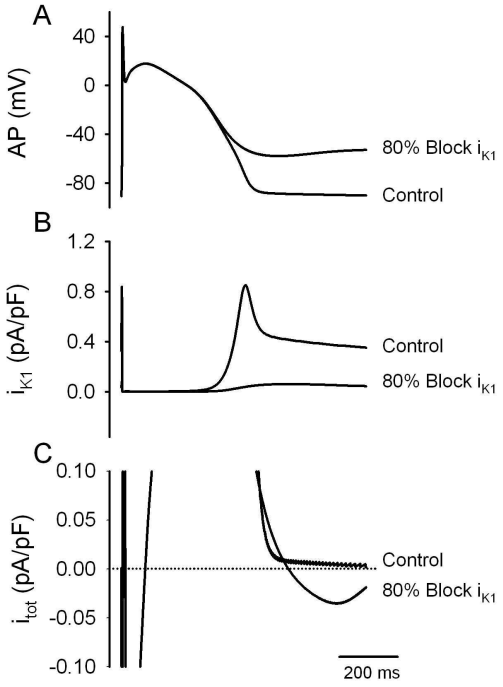

Figure 1. Action potentials generated by a supra-threshold stimulus applied to PB human ventricular cell model under control and 80% i_{K1} block conditions. Reduction of i_{K1} resulted in a more depolarized and unstable resting potential. (A) Action potentials. (B) i_{K1} during action potential. (C) Total net current during action potential. Dotted line marks the level of zero current.

An unstable resting potential by blocking i_{K1} can lead to spontaneous action potentials leading to rhythmic pacemaker activity by homoclinic bifurcation [5]. Further reducing i_{K1} increased the inward total net current during the final phase of AP repolarisation and the following phase of depolarisation. As a result, MDP was lifted more towards positive potential. By 85% reduction of i_{K1}, the net inward current became large enough to bring the cell membrane potential close to the take-off potential (about -60 mV) (Figure 2), around which sodium channel activation produced a large inward i_{Na} as indicated by the arrow in the figure. Once activated, i_{Na} continued to depolarize cell membrane producing a rapid upstroke. When the activation potential of L-type calcium channel was reached (around -45 mV), inward i_{CaL} contributed to complete a full action potential. In this way, regular auto-rhythmic pacemaker activity was established.

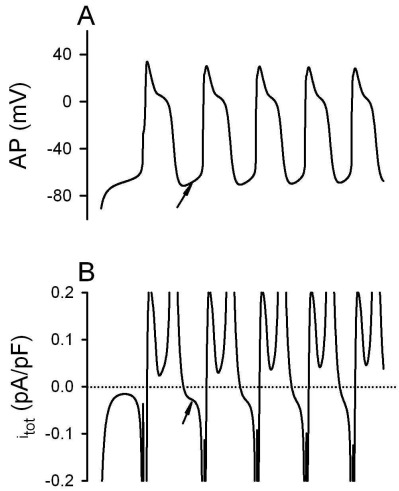

Figure 2. Auto-rhythmic electrical activity generated by 85% block of i_{K1} in the PB model. (A) Action potentials. (B) Total net current during action potential. Arrow marks the take-off potential, which activated a large inward i_{Na} responsible for a rapid AP upstroke.

However, the pacemaker activity is unstable, as it does not persist with time, as shown in Figure 3. In the figure, after t about 10s, the pacemaker activity terminated.

In order to understand the mechanisms underlying the self-termination of pacemaker activity, we studied the time courses of individual ion channel currents and ion concentrations. It was found that the self-termination of pacemaker activity was due to an accumulating increase in the intracellular Ca^{2+} concentration during the time course of the train of action potentials. With time, both the diastolic and systolic values of intracellular Ca^{2+} concentration drifted up, in a similar way as observed experimentally in cardiac myocytes undergoing a series of rapid electrical excitations [3]. With increased intracellular Ca^{2+} concentration, the L-type Ca channel is more inactivated through the mechanism of Ca dependent inactivation of Ca channel [6]. This produced an attenuating (damping) L-type calcium channel current (Figure 3B). When the amplitude of L-type Ca current decreased to a critical value, it failed to support AP up stroke. As a result, an in-completed action potential (marked by arrow) was produced leading to termination of pacemaker activity.

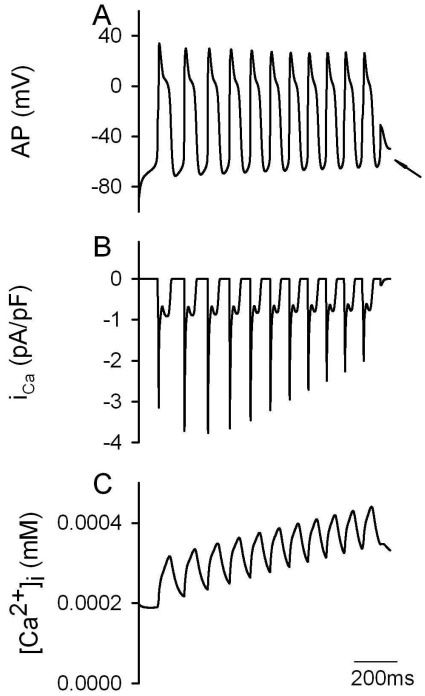

Figure 3. Self-termination of pacemaker activity in the PB model due to accumulating increase in the intracellular Ca^{2+} concentration resulting in damping i_{CaL} via Ca^{2+} dependent inactivation of L-type Ca^{2+} channel. (A) Action potentials. (B) Time course of i_{CaL}. (C) Time course of intracellular Ca^{2+} concentration.

The role of intracellular Ca^{2+} handling in determining the stability of the pacemaker activity was further investigated. Simulations were performed using the PB model with the same conditions as used in Figure 3 but with intracellular Ca^{2+} concentration buffered to a constant level of 250 nM. The result was shown in Figure 4. With a constant intracellular Ca^{2+} level, the accumulating inactivation of the L-type ionic channel during a series of APs was prevented. As a consequence, i_{CaL} was stable and a stable pacemaker activity was established.

It requires a large size for pacemaking tissue to overcome the suppressive electrotonic interaction from the surrounding quiescent cardiac tissue [7]. Using the 1D model, we have quantified the critical diameter of the pacemaker to be 14.4 mm. Below this, the pacemaker activity was suppressed by the surrounding ventricular muscle (Figure 5A); above which the pacemaker was able to pace and drive the surrounding tissue (Figure 5B).

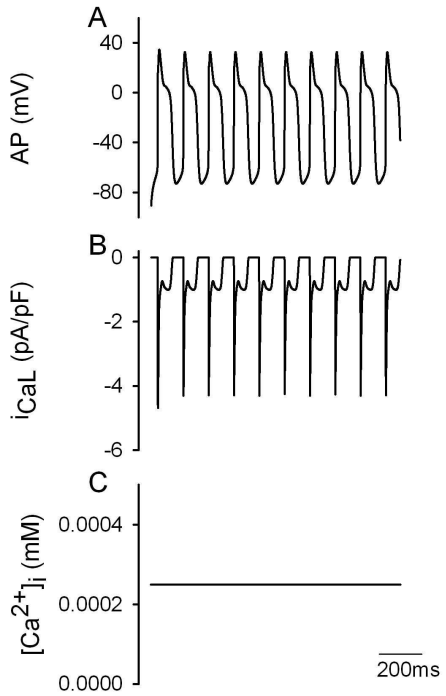

Figure 4. Stable pacemaking activity when intracellular Ca^{2+} level was buffered to a constant 250 nM. (A) Action potentials. (B) Time course of i_{CaL}. (D) Intracellular Ca^{2+} concentration.

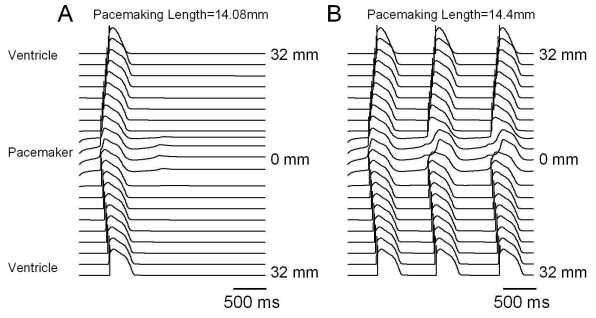

Figure 5. Space (vertical) and time (horizontal) plot of initiation and conduction of pacemaker activity in 1D model of ventricular strand with pacemaker size (A) below a critical size; (B) above a critical size.

4. Discussion and conclusions

In conclusion, depressing i_{K1} generates an inward total net current in the diastolic phase which is responsible for the spontaneous electrical activity in the genetically engineered pacemaker cells. This activity can be unstable and self-terminating, and intracellular Ca^{2+} handling plays an important role. In intact tissue model a critical size of engineered pacemaker is necessary for initiation and conduction of action potentials.

These conclusions are model independent. Similar results have been obtained using several other biophysically detailed models [5].

Acknowledgements

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