Role of I_f (Funny Current) on Biological Pacemaker – Insight From A Ventricular Model

Yacong Li[1], Kuanquan Wang[1], Qince Li[1], Xiangyun Bai[1], Henggui Zhang[1,2,3,4]

[1] School of Computer Science and Technology, Harbin Institute of Technology, Harbin, China
[2] School of Physics and Astronomy, the University of Manchester, Manchester, UK
[3] Space Institute of Southern China (Shenzhen), Shenzhen, China
[4] Key Laboratory of Medical Electrophysiology, Ministry of Education, Collaborative Innovation Center for Prevention and Treatment of Cardiovascular Disease/Institute of Cardiovascular Research, Southwest Medical University, Luzhou, China

Abstract

Biological pacemaker has been proposed as a promising alternative to electrical pacemaker for treating patients with impaired native cardiac pacemaker. It has been verified that overexpressing the hyperpolarization-activated funny channel current (funny current, I_f) can induce automatic pacing activities in ventricular myocytes (VMs). In this study, possible roles of I_f expression in generating automaticity in VMs are systematically investigated. We implemented a computational approach to study mechanism by which increase of I_f induces automaticity in VMs. The TP06 model of the electrical action potential in the human VMs was modified by incorporating I_f formulation. When the channel conductance of I_f was greater than 0.09 nS/pF, the VMs showed spontaneous pacing activities. During the time course of spontaneous action potentials, incorporating I_f induced changes in the intracellular ionic concentrations, leading to accumulation of intracellular calcium ($[Ca^{2+}]_i$) as well as sodium concentration ($[Na^+]_i$), but a decrease in the intracellular potassium concentration ($[K^+]_i$). By clamping $[Na^+]_i$ and $[K^+]_i$ to a constant level, a fast and stable spontaneous pacemaking activity was observed. This study helps to understand the mechanism of creating biological pacemakers using I_f overexpression.

1. Introduction

Although electrical pacemaker has saved millions of lives since it was used clinically, plenty of patients could not benefit from electrical pacemaker due to its high risk of open-chest surgery and the complications of implanting device [1]. Biological pacemaker is an innovative therapy as a replacement of electrical pacemaker. By injecting pacemaker cells into certain regions of the heart, such as atrium or ventricle, biological pacemaker therapy is expected to induce the sinoatrial-node-like pacing activity in the injected region and then drive the whole heart. The first issue of this method is producing pacemaker cells.

Previous experiments showed that overexpressing hyperpolarization-activated current (funny current, I_f) in nonautomatic cardiac cells could induce spontaneous automaticity in cells [2, 3]. Generally, there are three approaches to produce pacing cells: gene therapy, cell therapy and the hybrid therapy [1]. By gene therapy I_f ionic channels are expressed in cardiac myocytes by fusing certain gene into cells. For instance, injecting hyperpolarization-activated cyclic nucleotide–gated 2 (HCN2) that codes the I_f channel into canine left atrium [2] and left bundle-branch [3] via adenoviral constructs has successfully induced auto-rhythmicity in infected cells. Also, it has been shown that overexpressing HCN2 in two ventricular myocytes (VMs) system facilitated propagation of electrical signal from a HCN2-injected pacemaker cell to a quiescent cell [4]. With reprogramming the T-box 18 (TBX18), a kind of embryonic transcription factor related to the expression of HCN gene family in VMs, a superior gene therapy enabled the porcine heart to support normal heart rate and physical activity due to an increased I_f [5]. That study, for the first time, demonstrated autorhythmic biopacemaker in big animal heart. Cell therapy guide stem cells to differentiate into cardiomyocytes with pacemaker activity and then infects pacing cells into certain cardiac region. Embryonic Stem Cells (ESCs) [6], Mesenchymal Stem Cells (MSCs) [7] and Adipose Derived Stem Cells (ADSCs) [8] are common targets that is used to produce

 ISSN: 2325-887X DOI: 10.22489/CinC.2018.193

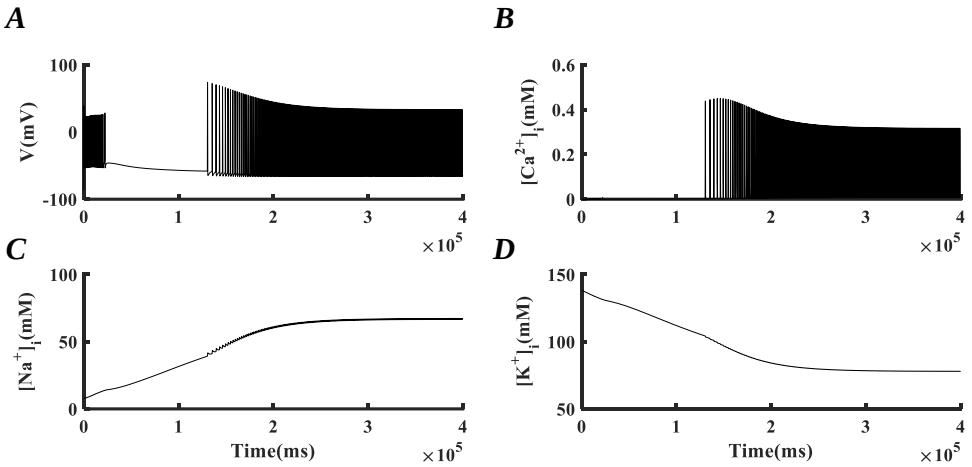

Figure 1. The action potential, $[Ca^{2+}]_i$, $[Na^+]_i$ and $[K^+]_i$ with time when G_f = 0.2 nS/pF.

biological pacemakers. For producing a preferable pacing capacity, a hybrid therapy is used, by which cardiomyocytes differentiated from stem cells are often injected with pacing-related gene, especially HCN gene [7, 8]. These experiments proved the feasibility of producing pacemaker cells based on non-autorhythmic cells, especially VMs, by expressing I_f current.

Although plenty of biological experiments have shown the necessity of expressing I_f to induce autorhythm [2, 3, 5] in VMs, the causal relationship between I_f expression and pacing activity is not clear. Also, the mechanism responsible for I_f-induced spontaneous pacing activity is incompletely understood. In this study, we modified the ventricular single cell model (TP06 [9]) to simulate the autorhythm induced by expressing I_f in VMs. An interesting phenomenon was an accumulation of intracellular calcium concentration ($[Ca^{2+}]_i$) when incorporating I_f into TP06 model. Meanwhile, the intracellular sodium and potassium concentration ($[Na^+]_i$ and $[K^+]_i$) also changed. Our simulation shows that the amplitude of I_f is a crucial factor that influence pacing ability. Finally, we demonstrated that a stable pacemaking cell model can be produced by clamping $[Na^+]_i$ and $[K^+]_i$.

2. Methods

Based on previous experiments [2, 3, 5], an autonomically responsive pacemaking function was produced by inclusion I_f in the TP06 model. In the model, the electrophysiological behavior of a single cell is described by the following ordinary differential equation

$$\frac{dV}{dt} = -\frac{I_{ion}}{C_m} \tag{1}$$

where V is voltage across cell membrane surfaces, t time, I_{ion} the sum of all transmembrane ionic currents, and C_m cell capacitance.

I_{ion} is given by the following equation

$$\begin{aligned} I_{ion} = {} & I_{Na} + I_{K1} + I_{to} + I_{Kr} + I_{Ks} + I_{CaL} + I_{NaCa} \\ & + I_{NaK} + I_{pCa} + I_{pK} + I_{bCa} + I_{bNa} + I_f \end{aligned} \tag{2}$$

Particularly, the formulation of I_f [10] is as follows

$$I_f = I_{f,Na} + I_{f,K} \tag{3}$$

$$I_{f,Na} = G_{f,Na} y (V - E_{Na}) \tag{4}$$

$$I_{f,K} = G_{f,K} y (V - E_K) \tag{5}$$

Where $G_{f,Na}$ and $G_{f,K}$ are maximal $I_{f,Na}$ and $I_{f,K}$ channel conductance, y is a time-independent inward rectification factor that is a function of voltage, E_{Na}, E_K are equilibrium potentials of Na^+ and K^+ channels respectively. In our simulation, $G_{f,Na}$ and $G_{f,K}$ share a same value, which is defined as G_f.

Formulations for other channel currents of I_{ion} are listed in ref. 9.

3. Results

3.1. Variations in intracellular ionic dynamics while incorporating I_f

Automatic pacing activity in the TP06 model after incorporation of I_f with G_f = 0.2 nS/pF as shown in Fig 1. The pacemaking action potential reached a steady state after 200 s (Fig. 1A). However, the cycle length (CL) was too long for potential clinical applications (2236 ms vs 1000 ms in normal human VMs). Note that during the first 100 s, the pacemaking action potential terminated after 26 pacing cycles, which was caused by drafting in the intracellular calcium concentration ($[Ca^{2+}]_i$), as well as Na^+ and K^+ concentrations. After 130 s, spontaneous activity resumed and gradually became stable.

Remarkably, $[Ca^{2+}]_i$ showed a step-wise change (Fig.1 B), which could be explained by the altered calcium dynamics induced by adding I_f. Calcium pumps in the

membrane of the sarcoplasmic reticulum (SR) (I_{up}) remained a high value for a long time, pumping excess calcium into the SR. Especially in silent duration, the Ca^{2+} in SR gradually accumulated. When resuming pacing activity, ryanodine receptors sensed this elevation of calcium in the SR and responded with a sudden increased release of calcium from the SR (I_{rel}). Through diffusion (I_{xfer}), the increased calcium released in the subspace travels to the cytoplasm. All of these contributed to a sudden influx of calcium ions into cytoplasm.

Except $[Ca^{2+}]_i$, other ionic concentration dynamics were also changed. Under the effect of I_f, I_{NaCa} presented a substantial rise, which resulted in an increase of $[Na^+]_i$ to 6.3 times bigger than the normal value in VMs (10.56 mM). At the same time, a rise of slow delayed rectifier current (I_{Ks}) and inward-rectifier potassium current (I_{K1}) as well as a soar of transient outward current (I_{to}) resulted a decrease of $[K^+]_i$ to ~60% of the normal value in VMs (134.8 mM). The changes of $[Na^+]_i$ and $[K^+]_i$ with time are shown in Fig. 1C and D.

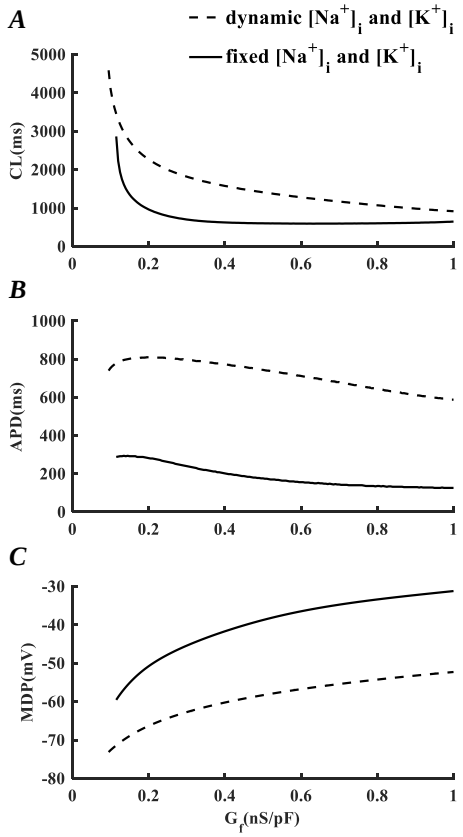

Figure 2. Measured CL, APD, MDP after stable solution (pacemaking cycle n=1500) with the change of G_f under dynamic $[Na^+]_i$ and $[K^+]_i$ (dotted line) and fixed $[Na^+]_i$ and $[K^+]_i$ (solid line). G_f is from 0 nS/pF to 1.0 nS/pF with 0.005 nS/pF increments. Under dynamic $[Na^+]_i$ and $[K^+]_i$, when G_f < 0.09 nS/pF, no auto rhythm presents. Under fixed $[Na^+]_i$ and $[K^+]_i$, when G_f < 0.11 nS/pF, no auto rhythm presents.

3.2. Effect of I_f density on pacing ability

In simulations, G_f was changed systematically to investigate the effect of I_f amplitude on pacing ability. The value of G_f was changed from 0 nS/pF to 1.0 nS/pF with a 0.005 nS/pF increment. When G_f < 0.09 nS/pF, autorhythm could not be induced in VMs. When $G_f \geqslant 0.09$ nS/pF, spontaneous rhythms showed in VMs model, which indicated the threshold of expressing I_f to produce a bio-pacemaker. Variations of characteristics of action potential with the change of G_f are shown in Fig. 2 (dotted line). With the rise of G_f, the CL gradually decreased and reached at approximately 1000 ms, while the action potential duration (APD) was almost linearly declining. As expected, the maximum diastolic potential (MDP) became more positive with the increase of G_f as massive I_f increased the automatic depolarization in phase 4, leading to a more positive MDP.

3.3. An optimized I_f-induced pacemaker by fixing $[Na^+]_i$ and $[K^+]_i$

As shown in above, the long CL was caused by the dramatically altered $[Na^+]_i$ and $[K^+]_i$. Further simulations were conducted by clamping $[Na^+]_i$ and $[K^+]_i$ to their original values (7.67 and 138.3 mM respectively [9]). Results are shown in Fig. 3, demonstrating a considerably improved pacing ability of pacemaker (Fig. 3, solid line). With G_f = 0.2 nS/pF, compared to dynamic $[Na^+]_i$ and $[K^+]_i$, the pacing activity reached a stable state more quickly. The CL and APD declined considerably (942 ms vs 2238 ms, and 290 ms vs 884 ms), and MDP was more positive (-50.44 mV vs -66.09 mV), suggesting the pacemaker was easier to depolarize (Fig. 3B, C, D). This was because the controlled $[Na^+]_i$ and $[K^+]_i$ suppressed the Na-K pump current (I_{NaK}), making depolarization easier, thus showing a stronger pacing ability.

Furthermore, we simulated the membrane potential with fixed $[Na^+]_i$ and $[K^+]_i$ when the value of G_f was changing from 0 nS/pF to 1.0 nS/pF with 0.005 nS/pF increments. The CL, APD, and MDP with the change of G_f are shown in Fig. 2 (solid line). When $[Na^+]_i$ and $[K^+]_i$ were fixed, the tendency of CL, APD, and MDP variations was similar to that under dynamic $[Na^+]_i$ and $[K^+]_i$, but the CL and APD was considerably shorter and the MDP was more positive, which meant a superior and robust pacing activity.

4. Conclusion

In this study, we simulated the automaticity induced by expressing I_f in VMs and demonstrated that overexpressing I_f could induce spontaneous beatings in a model of VMs. In the VMs model, after reaching the threshold of I_f, automaticity could be induced and the action potential became stable after a period.

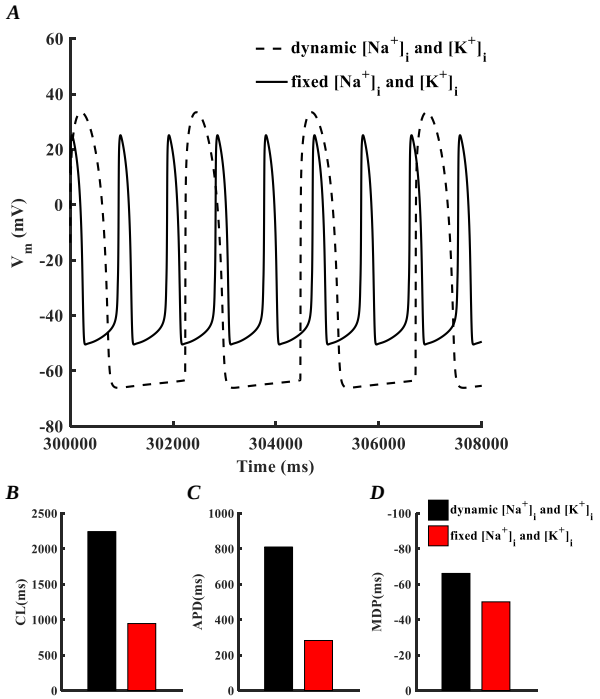

Figure 3. A: The action potential with time when G_f = 0.2 nS/pF under dynamic and fixed $[Na^+]_i$ and $[K^+]_i$. B: The CL in Fig. A. C: The APD in Fig. A. C: The MDP in Fig. A.

However, automaticity due to the action of I_f in VMs changed intracellular ionic dynamics, especially the accumulation of $[Ca^{2+}]_i$. Meanwhile, accumulation of $[Na^+]_i$ and a decrease of $[K^+]_i$ were also observed which led to a long CL. After clamping $[Na^+]_i$ and $[K^+]_i$ to original value, an improved biopacemaker was produced. In conclusion, these simulation results support the possibility of producing pacemakers based on VMs by overexpressing I_f. Possible effect of I_f amplitude on spontaneous beatings was also investigated. This study provides insights into understandings of biological pacemaker.

Acknowledgements

The work is supported by the National Natural Science Foundation of China (NSFC) under Grant nos.61572152, 61601143, 61571165 and 81770328, the Science Technology and Innovation Commission of Shenzhen Municipality under Grant nos. JSGG20160229125049615 and JCYJ20151029173639477, and Heilongjiang and China Postdoctoral Science Foundation under Grant nos.2015M581448.

References

[1] Rosen MR. Gene therapy and biological pacing. New Engl J Med 2014;371:1158-1159.

[2] Qu JH, Plotnikov AN, Danilo P, Shlapakova I, Cohen IS, Robinson RB, Rosen MR. Expression and function of a biological pacemaker in canine heart. Circulation 2003;107:1106-1109.

[3] Plotnikov AN, Sosunov EA, Qu JH, Shlapakova IN, Anyukhovsky EP, Liu LL, Janse MJ, Brink PR, Cohen IS, Robinson RB, Danilo P, Rosen MR. Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. Circulation 2004;109:506-512.

[4] Valiunas V, Kanaporis G, Valiuniene L, Gordon C, Wang HZ, Li L, Robinson RB, Rosen MR, Cohen IS, Brink PR. Coupling an hcn2-expressing cell to a myocyte creates a two-cell pacing unit. J Physiol-London 2009;587:5211-5226.

[5] Hu YF, Dawkins JF, Cho HC, Marban E, Cingolani E. Biological pacemaker created by minimally invasive somatic reprogramming in pigs with complete heart block. Sci Transl Med 2014;6

[6] Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 2001;108:407-414.

[7] Potapova I, Plotnikov A, Lu ZJ, Danilo P, Valiunas V, Qu JH, Doronin S, Zuckerman J, Shlapakova IN, Gao JY, Pan ZM, Herron AJ, Robinson RB, Brink PR, Rosen MR, Cohen IS. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. Circ Res 2004;94:952-959.

[8] Yang J, Song T, Wu P, Chen YJ, Fan XR, Chen H, Zhang J, Huang CX. Differentiation potential of human mesenchymal stem cells derived from adipose tissue and bone marrow to sinus node-like cells. Mol Med Rep 2012;5:108-113.

[9] ten Tusscher KH, Panfilov AV. Alternans and spiral breakup in a human ventricular tissue model. American journal of physiology. Heart and circulatory physiology 2006;291:H1088-1100.

[10] Zhang H, Holden AV, Kodama I, Honjo H, Lei M, Varghese T, Boyett MR. Mathematical models of action potentials in the periphery and center of the rabbit sinoatrial node. American journal of physiology. Heart and circulatory physiology 2000;279:H397-421.

Address for correspondence.

Henggui Zhang
Room 3.07, Shuster building
Manchester, M13 9PL, UK
henggui.zhang@manchester.ac.uk