An Engineering-Optimized Cardiac Pacemaker by Manipulating Na^+/Ca^{2+} Exchange Current and Na^+/K^+ Pumping Current

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Abstract

Biological pacemaker is a possible therapy for arrhythmias but there are several problems when creating single pacemaker cells based on ventricular myocytes by inhibiting inward rectifier current (I_{K1}) and combining hyperpolarization-activated funny channel current (I_f), such as the change of intracellular concentration equilibrium and un-physiological pacing frequency. Previous biological studies suggested that Na^+-Ca^{2+} exchange current (I_{NaCa}) -related gene increased 4-fold in pacemaker cells than normal cardiac myocytes. In addition, the accumulation of intracellular Na^+ was observed due to the combination of I_f and prompting feedback mechanism of Na^+/K^+ pumping may accelerate pumping out excessive Na^+ in pacemaker cells. In this study, we construct a pacemaker model based on a ventricular myocyte model by manipulating I_{K1} and I_f and optimize this pacemaker model by augmenting I_{NaCa} and I_{NaK}. Simulating results showed that overexpressing I_{NaCa} and I_{NaK} balanced the equilibrium of intracellular ionic concentrations effectively and enhanced the pacemaking ability. And the most optimized cooperation between I_{NaCa} and I_{NaK} was defined in this study. The action of I_f in pacemaker even changed in optimized model and the deep reason is illustrated in detailed. This study might guide the clinical research of biological pacemaker.

1. Introduction

Biological pacemaker is presented as an alternative therapy for pacemaker dysfunction, which believed to have the ability of overcoming the drawbacks of electronic pacemaker such as surgery risk, fixed size, and single rhythms [1]. Pacemaker cells could be induced from cardiac myocytes (CMs) or stem cells by gene therapy [2-4] as well as cell therapy [5-7]. It has been verified that pacemaker cells could initiate spontaneous beatings in co-cultured CMs [6, 7] by which open-chest surgery could be avoided. A superiority of bio-pacemaker is that it could respond to natural emotion [8]. Biological pacemaker experiments indicated that manipulating three kinds of gene could initiate spontaneous beatings in CMs: overexpressing hyperpolarization-activated funny channel current (I_f) related gene (such as HCN gene family [2, 6, 7]), suppressing inward rectifier current (I_{K1}) related gene (such as Kir2 gene [3]) or expressing TBX18 [4, 5] which is a transcription factor that operates the expression of I_f and I_{K1}.

In this study, we simulate an I_{K1}-I_f induced biological pacemaker cell model based on a ventricular myocytes (VMs) model [9] by suppressing I_{K1} and incorporating I_f. However, similar to the arrhythmia which was witnessed in biological experiments when overexpressing HCN gene [10, 11], the combination of I_f appeared to inhibit pacemaking activity in our pacemaker model. The possible reason of prolonged pacemaker's cycle length (CL) is the long diastolic interval (DI) and the change of intracellular ionic concentration equilibrium. According to a biological experiment, the expression of Na^+-Ca^{2+} exchange current (I_{NaCa})-related gene increased 4-fold in cardiac pacemaker cells differentiated from embryonic stem cells than that in normal CMs, and the pacing ability of pacemaker was dramatically stronger than control [12]. Theoretically, I_{NaCa} is an inward current which promotes depolarization during DI, thus overexpressing I_{NaCa} probably make contribution to shorten pacemaker's CL. As for the intracellular ionic concentration, our simulation indicated that the combination of I_f led to an accumulation of intracellular Na^+, so overexpressing Na^+/K^+ pumping (I_{NaK}) which could pump out extra intracellular Na^+ might have a positive effect on the equilibrium of intracellular ionic concentration. The optimal ratio between I_{NaK} and I_{NaCa} was defined as our optimized pacemaker model. Based on

 ISSN: 2325-887X DOI: 10.22489/CinC.2019.016

the optimized model, we illustrate the deep reasons of why the increase of I_{NaK} and I_{NaCa} promoted pacemaking activity and even changed the effect of I_f on pacemaker.

2. Methods

According to original VMs model [9], the electrophysiological behavior of a single pacemaker cell could be described by the following ordinary differential equation

$$\frac{dV}{dt} = -\frac{I_{ion}}{C_m} \quad (1)$$

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

The suppression of I_{K1} was simulated by decreasing the maximum conductance of I_{K1} (G_{K1}). And I_f formulation [13] was incorporated into VMs model to simulate the overexpression of I_f. Various I_f densities were simulated to illustrated its action in pacemaking ability by changing the maximum conductance of I_f (G_f). To optimize the original pacemaker, the formulations of I_{NaCa} and I_{NaK} were overexpressed by multiplying a coefficient (c_{NaCa} and c_{NaK} respectively). As a result, I_{ion} could be described by

$$I_{ion}=I_{Na}+I_{K1}+I_{to}+I_{Kr}+I_{Ks}+I_{CaL}+c_{NaCa}*I_{NaCa} +c_{NaK}*I_{NaK}+I_{pCa}+I_{pK}+I_{bCa}+I_{bNa}+I_f \quad (2)$$

The formulation of I_f is listed in Ref. [13] and the formulations of other ionic currents could be referenced in [9].

To illustrate the contribution of inward ionic currents to depolarization, we defined the normalized integral currents during early diastolic interval which started from the time at maximum diastolic potential (MDP) and lasted for 200 ms (the period between dashed line in Fig. 1) as the contribution value whose unit is pA/pF.

3. Results

3.1. The Effect of Overexpressing I_{NaCa} On Pacemaker

There were some inward ionic currents (such as I_{Na}, I_{CaL}, I_{NaCa}, I_f) which possibly promoted the depolarization of action potential (AP) during the early period of DI. The integral results showes that except pacemaking current – I_f, I_{NaCa} had the greatest contribution to depolarization (Fig. 1). Accordingly, the increase of I_{NaCa} might helped to reduce CL. Exactly, overexpressing I_{NaCa} could shorten CL slightly although it still could not reach biologically-sound pacing frequency (Fig. 2, five times increase of I_{NaCa} shortened CL by only 8%), because this inward current inhibited the repolarization of AP and prolonged action potential duration (APD).

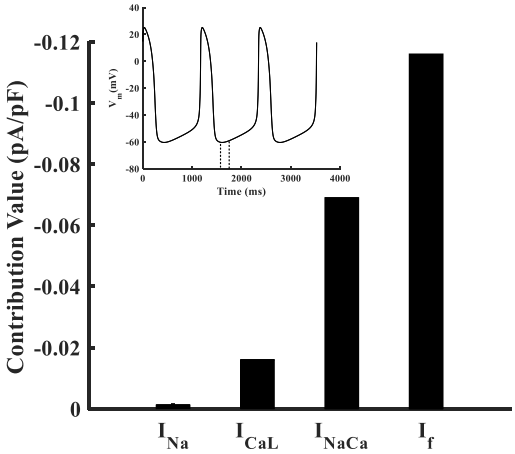

Figure. 1 The contribution value (Normalized integral currents during period between dashed line) of I_{Na}, I_{CaL}, I_{NaCa} and I_f during early DI.

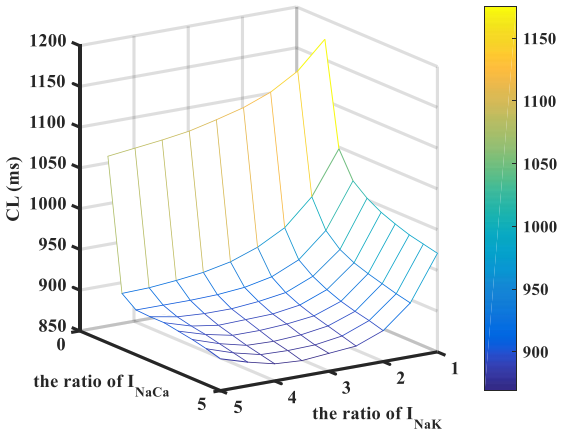

Figure. 2 The cycle length (CL) under different ratio of I_{NaK} and I_{NaCa} when the ratio of I_{K1} and I_f is 0.02 and 1 respectively.

3.2. The Effect of Overexpressing I_{NaK} On Pacemaker

According to gene therapy experiments [10, 11], acute I_f may cause arrhythmia in pacemaker. In our pacemaker model, the change of intracellular ionic concentration equilibrium appeared due to additional I_f. More specifically, the expression of I_f in single VMs model caused accumulation of intracellular Na^+ by a large extent. As a result, I_{NaK} was overexpressed to discharge extra intracellular Na^+. The overexpression of I_{NaK} could also decrease maximum diastolic potential and promote the activation of I_{Na}. Indeed, the increase of I_{NaK} helped to reduce $[Na^+]_i$ and had a positive effect on the equilibrium of intracellular ionic concentration (Figures did not show).

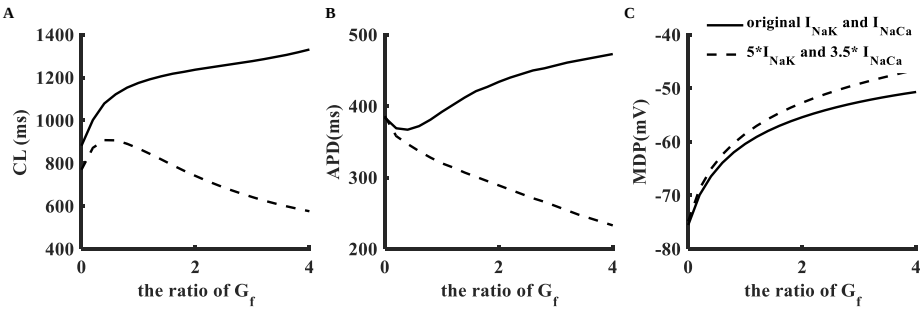

Figure. 3 The cycle length (CL), action potential duration (APD) and maximum diastolic potential (MDP) after stable (n=1500) under original I_{NaK} and I_{NaCa} (solid line) as well as 5 times I_{NaK} and 3.5 times I_{NaCa} (dashed line) with the change of I_f from 0 to 4 times in 0.2 increments when the ratio of I_{K1} is 0.02.

However, the CL went down slightly (Fig. 2, five times increase of I_{NaK} decreased CL by 17%) which was not sufficient to create a qualified pacemaker.

3.3. The Combined Action of Overexpressing Both I_{NaCa} and I_{NaK} On Pacemaking Activity

Due to the weak action of overexpressing single current, we attempted to overexpress I_{NaK} and I_{NaCa} conjunctly to improve pacemaking activity. Simulation result shows that when I_{NaK} and I_{NaCa} was 5 and 3.5 times respectively, the CL decreased considerably (Fig. 2). We defined 5 times I_{NaK} and 3.5 times I_{NaCa} as case 2 and the original pacemaker model (I_{NaK} and I_{NaCa} was 1 times) as case 1 to analyze the pacing mechanism.

We calculated the CL and APD of pacemaker with different G_f in both cases when G_{K1} was suppressed to 2% of original value (Fig. 3, solid line is case 1 and dashed line is case 2). Apparently, the CL in case 2 was much less than that in case 1. To explain the deep reasons, the action potential, L-type calcium current (I_{CaL}), intracellular calcium concentration ($[Ca^{2+}]_i$), I_{NaCa}, I_{NaK} and I_f in case 1 and case 2 under 1 time I_f and 1.6 times I_f are showed in Fig. 4. In case 2, I_{NaCa} during DI was much greater than that in case 1 (Fig. 4D), as the AP could depolarize more readily, which contributed to a short DI. Also, the increased I_{NaCa} promoted the discharge of intracellular Ca^{2+} (Fig. 4C), thus the I_{CaL} whose activated gate is calcium-dependent was less inhibited (Fig. 4B), which shortened the APD. In addition, the increased I_{NaK} (Fig. 4E) accelerated the outflow of intracellular Na^+ and maintained membrane potential at a negative level. As a result, the activation degree of I_f was greater in case 2 especially during the early DI (Fig. 4F), which helped the elevation of membrane potential effectively. These factors contributed to a shorter CL jointly.

3.4. The Change of I_f's Action in Optimized Pacemaker

The overexpression of I_{NaCa} and I_{NaK} changed I_f's action in pacemaking activity. With the increase of G_f, the CL went down in case 2 but went up in case 1 (Fig. 3A). In case 2, due to the rise of I_{NaCa}, the $[Ca^{2+}]_i$ was more than 10 times smaller than that in case 1 (Fig. 4C). At the same time, the calcium concentration in subspace ($[Ca^{2+}]_{SS}$) decreased. As a result, the calcium-dependent inactivated gate (f_{CaSS}) of I_{CaL} was not inhibited, thus the I_{CaL} was activated in a greater extent. Especially during DI, with the increase of I_f, the activation degree of I_{CaL} became larger in case 2 but did not changed in case 1 (Fig. 4B). Under the change of other ionic current such as I_{NaK}, the slope of depolarization during DI remained the same in case 2 (Fig. 4A, orange line) but slowed down in case 1 (Fig. 4A, blue line). This slight difference finally caused the different variation tendency of CL with the increase of I_f in these two cases.

4. Conclusion

As has been shown in Fig.3, transforming VMs cell into biological pacemaker by suppressing I_{K1} and incorporating I_f may change the equilibrium of intracellular concentration which resulted in a weaker pacemaking activity. In this study, we provide optimized approaches by increasing I_{NaCa} and I_{NaK} to improve our pacemaker model and find that the cooperation of these two currents shows the most satisfying results. Increasing I_{NaCa}, which is an inward current, accelerated the early depolarization considerably and shortened DI. Extra I_{NaCa} also promoted the outflow of intracellular Ca^{2+}, thus decreased its negative effect on the activation of I_{CaL}, finally narrowed APD. As for I_{NaK}, overexpressing I_{NaK} promoted the feedback mechanism and discharged extra intracellular Na^+ quickly. Also, it helped to repolarize membrane potential. Accordingly, I_f was activated in a greater extent and CL was shortened.

Compared with original pacemaker model, the tendency of CL with the increase of I_f changes completely in the optimized model. With the increase of I_f, CL became

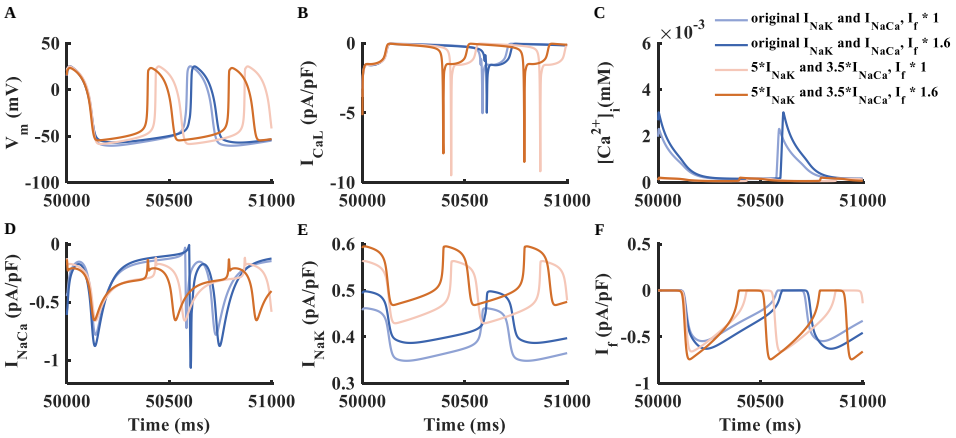

Figure. 4 The membrane potential (V_m), I_{CaL}, $[Ca^{2+}]_i$, I_{NaCa}, I_{NaK} and I_f with original (Blue line) and increased (orange line) I_{NaK} and I_{NaCa} under the ratio of I_f 1 (light line) and 1.6 times (deep line).

shorter in optimized model but longer in original model, which was due to the calcium dynamic equilibrium. The increase of I_{NaCa} and I_{NaK} maintained $[Ca^{2+}]_i$ at a normal level, so the calcium-dependent inactivated gate of I_{CaL} became more active. With I_f increasing, the depolarization rate remained the same in optimized model but slowed down in original model, which caused the different tendency of pacemaking activity with the increase of I_f.

In conclusion, this simulation provides an effective method to solve the problem of long CL induced by I_f incorporation, which could guide the clinical research of biological pacemaker.

Acknowledgements

The work is supported by the National Science Foundation of China (NSFC) under Grant Nos. 61572152 (to HZ), 61571165 (to KW), 61601143 (to QL) and 81770328 (to QL), and China Postdoctoral Science Foundation under Grant Nos. 2015M581448 (to QL).

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