An Engineering-Optimized Cardiac Pacemaker by Manipulating Na+/Ca2+ 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\textsubscript{K1}) and combining hyperpolarization-activated funny channel current (I\textsubscript{f}), such as the change of intracellular concentration equilibrium and un-physiological pacing frequency. Previous biological studies suggested that Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange current (I\textsubscript{NaCa}) -related gene increased 4-fold in pacemaker cells than normal cardiac myocytes. In addition, the accumulation of intracellular Na\textsuperscript{+} was observed due to the combination of I\textsubscript{f} and prompting feedback mechanism of Na\textsuperscript{+}/K\textsuperscript{+} pumping may accelerate pumping out excessive Na\textsuperscript{+} in pacemaker cells. In this study, we construct a pacemaker model based on a ventricular myocyte model by manipulating I\textsubscript{K1} and I\textsubscript{f} and optimize this pacemaker model by augmenting I\textsubscript{NaCa} and I\textsubscript{NaK}. Simulating results showed that overexpressing I\textsubscript{NaCa} and I\textsubscript{NaK} balanced the equilibrium of intracellular ionic concentrations effectively and enhanced the pacemaking ability. And the most optimized cooperation between I\textsubscript{NaCa} and I\textsubscript{NaK} was defined in this study. The action of I\textsubscript{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 cocultured 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\textsubscript{f}) related gene (such as HCN gene family [2, 6, 7]), suppressing inward rectifier current (I\textsubscript{K1}) related gene (such as Kir2 gene [3]) or expressing TBX18 [4, 5] which is a transcription factor that operates the expression of I\textsubscript{f} and I\textsubscript{K1}.

In this study, we simulate an I\textsubscript{K1}-I\textsubscript{f} induced biological pacemaker cell model based on a ventricular myocytes (VMs) model [9] by suppressing I\textsubscript{K1} and incorporating I\textsubscript{f}. However, similar to the arrhythmia which was witnessed in biological experiments when overexpressing HCN gene [10, 11], the combination of I\textsubscript{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\textsuperscript{+}-Ca\textsuperscript{2+} exchange current (I\textsubscript{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\textsubscript{NaCa} is an inward current which promotes depolarization during DI, thus overexpressing I\textsubscript{NaCa} probably make contribution to shorten pacemaker’s CL. As for the intracellular ionic concentration, our simulation indicated that the combination of I\textsubscript{f} led to an accumulation of intracellular Na\textsuperscript{+}, so overexpressing Na\textsuperscript{+}/K\textsuperscript{+} pumping (I\textsubscript{NaK}) which could pump out extra intracellular Na\textsuperscript{+} might have a positive effect on the equilibrium of intracellular ionic concentration. The optimal ratio between I\textsubscript{NaK} and I\textsubscript{NaCa} was defined as our optimized pacemaker model. Based on
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

$$\frac{dV}{dt} = \frac{I_{ion}}{C_m}$$ (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 illustrate 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, $I_{NaK}$ could be described by

$$I_{ion} = I_{Na} + I_{K1} + I_{Kr} + I_{Kr} + I_{CaL} + c_{NaCa} * I_{NaCa} + c_{NaK} * I_{NaK} + I_{pCa} + I_{Na} + I_{Na} + I_f$$ (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).

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$^+$], and had a positive effect on the equilibrium of intracellular ionic concentration (Figures did not show).
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 $G_I$ 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 $I_{CaL}$ in case 1 and case 2 under 1 time $I_{CaL}$ and 1.6 times $I_{CaL}$ 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 shorter 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 $I_I$ 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 $I_I$’s Action in Optimized Pacemaker

The overexpression of $I_{NaCa}$ and $I_{NaK}$ changed $I_I$’s action in pacemaking activity. With the increase of $G_I$, 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$^{2+}$])$_s$ decreased. As a result, the calcium-dependent inactivated gate ($I_{CaL}$) of $I_{CaL}$ was not inhibited, thus the $I_{CaL}$ was activated in a greater extent. Especially during DI, with the increase of $I_I$, 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 $I_I$ 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 $I_I$ 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, $I_I$ was activated in a greater extent and CL was shortened.

Compared with original pacemaker model, the tendency of CL with the increase of $I_I$ changes completely in the optimized model. With the increase of $I_I$, CL became...
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$^{2+}$] 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.

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References


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