Bifurcation analysis of a human ventricular myocyte model for biological pacemaker engineering

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Abstract: A biological pacemaker, which is a pacemaker cell created from normally quiescent non-pacemaking cells, is expected to be an alternative to electronic pacemakers. Recent studies on biological pacemaker engineering have revealed that a modification of ionic currents across the cell membrane elicits the pacemaking ability of a ventricular myocyte. We perform bifurcation analyses using an elaborate mathematical cell model to investigate an efficient way to create biological pacemakers from human ventricular myocytes. Pacemaker activity appears during the suppression of $I_{K1}$. Furthermore, we show that an additional increment of $I_{CaL}$, $I_{NaCa}$, or $I_f$ facilitates the generation of pacemaker activity under $I_{K1}$-reduced conditions.

Key Words: biological pacemaker, human ventricular myocyte model, bifurcation analysis

1. Introduction

The heart plays the role of a pump for blood circulation by regular repetition of contractions and relaxations. A periodic heartbeat is initiated by electrical signals (called action potentials) generated in the sinoatrial node, which is the pacemaker of a heart [1]. Its spontaneous pacemaker activity drives cardiac myocytes and induces cardiac contraction. Dysfunction of the sinoatrial node causes cardiac arrhythmias, including bradycardia or tachycardia, which require the implantation of electronic devices [2]. An electronic cardiac pacemaker is the only effective therapy for bradyarrhythmia to sustain the heart rate. However, this device has several limitations such as high cost, risks of bacterial infection, or mechanical failure [3].

Recent studies on biological therapies for arrhythmias have demonstrated that pacemaker activity is generated in a cardiomyocyte (which is an intrinsically quiescent non-pacemaking cell) by genetic modification of the ionic current on the cell membrane. These artificial pacemaker cells are called biological pacemakers, and are expected to be an alternative to conventional electronic pacemakers. Previous attempts to create a biological pacemaker by modification of specific ionic currents have focused on two general approaches [3, 4]. The first approach is the suppression of the inward rectifier potassium current ($I_{K1}$), which inhibits the ability of myocytes to generate spontaneous pacemaker activity [5]. The second is the insertion of the hyperpolarization-activated current ($I_f$) which is an
essential current for pacemaking in native pacemaker cells [6].

The interactions between the membrane potential and various ionic currents must be understood for biological pacemaker engineering, but these interactions are difficult to elucidate using only physiological experiments. Silva et al. [7] simulated the generation of the biological pacemaker activity by adjusting the $I_{K1}$ conductance in a guinea pig ventricular myocyte model and examined the responsiveness (rate variation) to β-adrenergic stimulation. The initiation mechanisms of pacemaking have also been investigated by bifurcation analyses of a guinea pig ventricular myocyte model [8] or a human ventricular myocyte model [9]. In the present paper, we analyze a human ventricular myocyte model, which is described by nonlinear ordinary differential equations. We explore the bifurcation structure of the model by changing the conductance of the ion channel to investigate methods to create a biological pacemaker from human ventricular myocytes.

This paper is organized as follows. In section 2, we introduce the human ventricular myocyte model. We also show the interrelations between the membrane potential and ionic currents during the action potential. In section 3, we show a method to induce pacemaker activity by changing single ionic currents based on one-parameter bifurcation analysis to change the conductance of the ion channel as the bifurcation parameter. Section 4 shows the effects of an additional ionic current on the pacemaker activity generated by the suppression of the inward rectifier potassium current $I_{K1}$. Finally, we provide our conclusions in section 5.

2. Reduced ten Tusscher–Noble–Noble–Panfilov model

Action potentials originating from the sinoatrial node are propagated to the whole heart and excite cardiomyocytes, resulting in a cardiac contraction. The action potential is the temporal variation of the membrane potential (the difference of the electric potential between the inside and the outside of the cell membrane) caused by ion transfer through ion channels on the cell membrane according to the electrochemical potential. Ion channels open and close depending on the membrane potential. Ions move in or out the cell membrane through these channels, consequently generating action potentials. Since the Hodgkin–Huxley model [10] was proposed in 1952, various mathematical models describing the electrical excitation of cardiac cells have been proposed according to animal species or regions of the heart [11]. In the present paper, we use the reduced ten Tusscher–Noble–Noble–Panfilov (TNNP) model [12], which is a human ventricular myocyte model described by Hodgkin–Huxley-type equations with nine variables. The reduced TNNP model is a simplified version of the original TNNP model [13, 14] constructed by removing some variables. Intracellular concentrations of Na$^+$, K$^+$, and Ca$^{2+}$ are treated as constants in the reduced TNNP model, but are variables in the TNNP model. Moreover, fast changing gating variables: the $r$ gate of $I_{to}$, the $x_{r2}$ gate of $I_{Kr}$, and the $d$ gate of $I_{CaL}$, are removed and replaced by functions that depend on the membrane potential ($r_{\infty}$, $x_{2\infty}$, and $d_{\infty}$ in the reduced TNNP model). The reduced TNNP model represents the electrochemical characteristics of the action potential generation in a human ventricular myocyte. The model behavior is described with the following differential equations:

\[
\frac{dV}{dt} = - (I_{stim} + I_{Na} + I_{CaL} + I_{to} + I_{Kr} + I_{Ks} + I_{K1}) \\
\frac{d\chi}{dt} = \frac{\chi(V)}{\tau_{\chi}(V)} - \chi, \quad (\chi = m, h, j, f_1, f_2, x_{r1}, x_s, s)
\]

where $V$ [mV] is the membrane potential, and $m$, $h$, $j$, $f_1$, $f_2$, $x_{r1}$, $x_s$, and $s$ are gating variables that represent the opening and closing dynamics of the ion channels. $I_{stim}$ [pA/pF] is the externally applied stimulus current. $I_{Na}$, $I_{CaL}$, $I_{to}$, $I_{Kr}$, $I_{Ks}$, $I_{K1}$, $I_{NaCa}$, $I_{pCa}$, $I_{pK}$, $I_{bNa}$, and $I_{bCa}$ [pA/pF] are the ionic currents, which represent the flow of ions through each ion channel. $\tau_{\chi}(V)$ and $\chi_{\infty}(V)$ are the time constant and the steady-state value of the gating variables, respectively. Both of which are functions that depend on the membrane potential. As an example, the fast Na$^+$ current $I_{Na}$ is given by the following equation:

\[
I_{Na} = c_{Na} G_{Na} m^3 h j (V - E_{Na})
\]

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where $G_{Na}$ [nS/pF] is the maximum conductance of ion channels and $E_{Na}$ [mV] is the equilibrium potential of Na$^+$. To simplify the bifurcation analysis, we include an additional coefficient $c_{Na}$ (standard value is 1.0) in the model. We changed the coefficients of the ionic currents as bifurcation parameters in the following analysis. The equations of the other ionic currents, $\tau_s(V)$, and $\chi_\infty(V)$ are summarized in appendix A.

Figure 1 shows an example of the solution of the model in the standard condition (all coefficients are 1.0), where the model is paced at 1 Hz with 1 ms stimuli of 60 pA/pF. Because a ventricular myocyte is normally quiescent, it keeps a constant membrane potential (resting potential). When a ventricular myocyte receives an extrinsic electrical stimulus at 3100 ms, the inward sodium current $I_{Na}$ and the subsequent inward calcium current $I_{CaL}$ increase sharply, contributing to the upstroke of the action potential (depolarization). After the depolarization, the outward currents cancel the inward currents, maintaining a constant membrane potential. Finally, the membrane is restored to the resting potential by both the increase in the outward potassium currents ($I_{Ks}$, $I_{K1}$, $I_{K1}$) and the decrease of $I_{CaL}$ (repolarization). During the resting potential, $I_{K1}$ balances with the inward currents, contributing to the stable resting potential.

### 3. Method to elicit pacemaker activity by changing the ionic current

In this section, we investigate a method to initiate pacemaker activity in human ventricular myocytes by the bifurcation analysis of the reduced TNNP model. Since the pacemaker activity of the cells is a periodic oscillation of the membrane potential, the periodic solutions of the membrane potential $V$ are searched while changing the coefficient $c_{ion}$ (ion=$Na$, $CaL$, to, $Kr$, $Ks$, $K1$, $NaCa$, $NaK$, PCa, PK) of each ionic current as a bifurcation parameter. $c_{CaL}$ and $c_{Na}$ are not checked because $I_{Ca}$ and $I_{Na}$ cannot be changed experimentally. ($I_{Ca}$ and $I_{Na}$ are the background currents.) In this paper, we use the bifurcation analysis software AUTO [15] to construct bifurcation diagrams. In the following analysis, we ignore the externally applied stimulus current ($I_{stim} = 0$).

#### 3.1 Inward rectifier potassium current $I_{K1}$

The first approach to create a biological pacemaker is the suppression of the inward rectifier potassium current $I_{K1}$. Miake et al. considered that normally quiescent ventricular myocytes may be excited spontaneously, but this ability is typically suppressed by $I_{K1}$, which strongly stabilizes the negative resting potential. They demonstrated that the inhibition of $I_{K1}$ by genetic modification produces a spontaneous pacemaker activity in guinea pig ventricular myocytes [5].

Figure 2 shows the one-parameter bifurcation diagram for the bifurcation parameter $c_{K1}$, which is the coefficient of $I_{K1}$. The membrane potential $V$ at the equilibrium points and the amplitude of periodic orbits are plotted for each value of the bifurcation parameter. HB, SN, and HC denote the bifurcation points of Hopf, saddle-node, and homoclinic bifurcations, respectively. The coordinates $(c_{K1},V)$ of the bifurcation points are as follows: HB1(−4.97, −19.78), HB2(0.010, −54.36), SN1(0.17, −34.04), SN2(0.010, −53.54), HC1(0.025, 24.92), HC2(0.010, −52.52). The coefficients of the ion channels can not be negative in intact myocytes. Figure 2(b) shows the positive parameter range in Fig. 2(a).

In the standard condition ($c_{K1} = 1.0$), only a stable equilibrium point corresponding to the resting membrane potential exists. When $c_{K1}$ is decreased to 0.17, unstable equilibrium points appear via saddle-node bifurcation SN1. Subsequently, periodic solutions appear via homoclinic bifurcation HC1 when $c_{K1}$ is reduced to 0.025. The unstable equilibrium point (0.025, −47.13) is the saddle point of HC1. The period and amplitude of the periodic orbits decrease with decreasing $c_{K1}$. Periodic solutions eventually disappear at supercritical Hopf bifurcation HB1. In addition, unstable periodic solutions emanated from subcritical Hopf bifurcation HB2 hit against the saddle point (0.010, −52.52) and disappear at HC2. The stable equilibrium point of the resting membrane potential is destabilized via HB2. Thus, both the stable equilibrium point and the stable periodic orbit coexist between HB2 and HC1. For each value of $c_{K1}$ between HB1 and HB2, only a stable periodic solution exist, which is an essential condition for stable pacemaking of a biological pacemaker. Figure 3 shows a typical scenario.
Fig. 1. Action potential simulated by the reduced TNNP model in standard condition ($c_{ion} = 1.0$). Top figure shows the membrane potential and the lower four figures show the ionic currents. Negative and positive values denote inward currents (flowing from the outside to the inside of the membrane) and outward currents, respectively.

Waveform of the membrane potential corresponding to stable periodic solutions. The membrane potential spontaneously oscillates without an extrinsic stimulus. Consistent with the results by Kurata et al. [9], the suppression of the inward rectifier potassium current $I_{K1}$ yields a spontaneous pacemaker activity in human ventricular myocytes. However, since $c_{K1}$ cannot take a negative value, the pacemaker activity is generated only within the range of $0 \leq c_{K1} \leq 0.01$. It would be difficult for an intact cell to keep $c_{K1}$ in such a narrow range to produce durable pacemaker activity.
3.2 L-type calcium current $I_{CaL}$

The possibility of the other ionic currents to generate the pacemaker activity is examined by changing their conductances. Figure 4 shows the one-parameter bifurcation diagram where the coefficient of the L-type calcium current $I_{CaL}$ is the bifurcation parameter. The coordinates $(c_{CaL}, V)$ of the bifurcation points are as follows: HB$(32.85, 0.39)$, SN1$(319.56, -79.38)$, SN2$(1.46, -28.20)$, DC$(29.54, 12.41)$, HC$(29.58, 58.39)$.

In the standard condition $(c_{CaL} = 1.0)$, only one stable equilibrium point corresponding to the resting potential exists. When $c_{CaL}$ is increased to 29.54, a pair of stable periodic and unstable periodic orbits emanate from double-cycle bifurcation. The stable periodic orbit converges on HB as $c_{CaL}$ is increased. At HC bifurcation, an unstable periodic orbit hits against the saddle point $(29.58, -55.97)$ and disappears. Stable periodic oscillations (pacemaker activity) are generated for the values of $c_{CaL}$ between DC and HB. For each value of $c_{CaL}$ between DC and HB, however, a stable equilibrium point coexists with a stable periodic solution, which may be unsuitable for a pacemaker. Figure 5 shows the time course of the membrane potential for two different initial values of the membrane potential (denoted by $V_0$) when $c_{CaL} = 30$. The waveform of the membrane potential in the steady state depends on the initial value. In addition, the values of parameter $c_{CaL}$ corresponding to the stable periodic solutions are too large.

We checked the other ionic currents, except $I_bCa$ and $I_bNa$. The oscillatory activity is also induced by changing the coefficient of $I_{NaCa}$, $I_{NaK}$, or $I_{pCa}$. In the case of $I_{NaCa}$, the pacemaker activity is induced by increasing $c_{NaCa}$ to a large parameter value, which is similar to the case of $I_{CaL}$. In the case of $I_{NaK}$ or $I_{pCa}$, the oscillation appears only with negative parameter values, which is inappropriate for biological pacemakers.
Fig. 4. One-parameter bifurcation diagram for $c_{CaL}$. (a) Global bifurcation structure. (b) Magnification of positive parameter range in Fig. 4(a). Solid and broken parts of the black curves denote stable and unstable equilibrium points, respectively. Solid and broken parts of the red curves denote the amplitude of stable and unstable periodic orbits, respectively. The bifurcation points of Hopf, saddle-node, homoclinic, double-cycle bifurcations are denoted by HB, SN, HC, and DC, respectively. Periods (ms) of several periodic orbits are also shown.

Fig. 5. Membrane potential waveform of the equilibrium point and the periodic orbit in Fig. 4. (a) $c_{CaL} = 30$, $V_0 = -84$. (b) $c_{CaL} = 30$, $V_0 = 10$.

4. Effects of additional ionic current on the generation of the pacemaker activity during $I_{K1}$ suppression

As shown in Figs. 2 and 3, spontaneous pacemaker activity appears during $I_{K1}$ suppression. However, the extremely limited values of $c_{K1}$ for the pacemaker activity suggest creation difficulty or the instability of biological pacing. Therefore, whether additional changes of another ionic current improve the drawbacks of the $I_{K1}$ reduction method is examined. In this section, we explore the coupling effects of pacemaking-induced currents by two-parameter bifurcation analyses, and investigate a more efficient method to generate the pacemaker activity.

4.1 L-type calcium current $I_{CaL}$ or Na$^+$/Ca$^{2+}$ exchanger current $I_{NaCa}$

At first, the effect of the L-type calcium current $I_{CaL}$ on the pacemaking under the $I_{K1}$ reduced condition is examined. Figure 6 shows a two-parameter bifurcation diagram where two coefficients $c_{K1}$ and $c_{CaL}$ are the bifurcation parameters. Each curve in the two-parameter bifurcation diagram designates the locus of the bifurcation point (bifurcation curve) or the periodic solution with specific period (contour line of period). The saddle-node bifurcation curve bends sharply at ($-0.011, 0.76$) (cusp bifurcation). The vertical and horizontal branches of the saddle-node bifurcation correspond to the SN2 and SN1 points in Fig. 2, respectively. The Hopf bifurcation curve emanates from the saddle-node bifurcation curve at ($0.034, 1.98$) and runs almost parallel to the horizontal axis. The two bifurcation curves of the Hopf bifurcation points (HB1 and HB2) overlap. The contour lines of the periodic solutions emanate from the Hopf bifurcation curve and run to the upper right along with
the saddle-node bifurcation curve SN2. A periodic oscillation appears in the upper side of the Hopf bifurcation curve and in the left side of the SN2 branch of the saddle-node bifurcation curve. Figure 7 shows a typical waveform of the membrane potential corresponding to the stable periodic solution. The SN2 branch running to upper right with the increase of $c_{CaL}$ demonstrates that the critical value of $c_{K1}$ for the biological pacemaker activity is extended by increasing $I_{CaL}$.

Figure 8 shows the two-parameter bifurcation diagram for $c_{K1}$ and $c_{NaCa}$. Similar to the case of $I_{CaL}$, as $c_{NaCa}$ increases, HB2 runs to the upper right of the figure along with SN2 and the $c_{K1}$ range for the pacemaker activity broadended.

4.2 Hyperpolarization-activated current $I_f$

Spontaneous pacemaker activity is initiated by a net inward current during the slow depolarization (diastolic depolarization) phase after repolarization. The diastolic depolarization increases the membrane potential to the threshold of the next action potential, generating continuous pacing. Hyperpolarization-activated current $I_f$, which is principally present in the sinoatrial node cell and absent in the ventricular myocyte, is activated by the hyperpolarized membrane potential (a negatively large potential after the repolarization) and flows inwardly during diastolic depolarization. Due to these properties, $I_f$ is called the “pacemaker current”, which plays an important role in the pacemaker activity of the sinoatrial node [16]. The effects of $I_f$ installation in a human ventricular myocyte during $I_{K1}$ suppression on biological pacemaker generation have been already discussed based a mathematical model [17]. In the previous study, however, incompleteness of the model of the
Fig. 8. Two-parameter bifurcation diagram for \( c_{K1} \) and \( c_{NaCa} \). (a) Global bifurcation structure. (b) Magnification of positive parameter range in Fig. 8(a). Red and blue curves designate the loci of Hopf and saddle-node bifurcation points, respectively. Gray shaded area shows the parameter range for the pacemaker activity.

\( I_f \) current due to the lack of experimental data from human cardiomyocytes might be a drawback. For an advanced investigation, we used the \( I_f \) current model constructed based on the experimentally recorded action potentials in the human sinoatrial node. Furthermore, we explored the bifurcation structure in more detail.

The Verkerk model [18] of the \( I_f \) current in human sinoatrial node is incorporated to the reduced TNNP model. (The original reduced TNNP model does not include \( I_f \) due to its small amplitude.) The Verkerk model is described using the Hodgkin–Huxley-type kinetic scheme:

\[
I_f = c_f G_f y(V - E_f) \quad (4)
\]

\[
\frac{dy}{dt} = \frac{y_\infty(V) - y}{\tau_y(V)} \quad (5)
\]

where \( y \) is the gating variable, and \( c_f \) is the conductance coefficient. Figure 9 shows the two-parameter bifurcation diagram where \( c_{K1} \) and \( c_f \) are the bifurcation parameters. Two saddle-node bifurcation curves SN1 and SN2 appear (SN2 and HB2 almost completely overlap). The two saddle-node curves approach each other as \( c_f \) increases and eventually disappear via cusp bifurcation (0.28, 52) (not shown in Fig. 9). The Hopf bifurcation curve emanates from the saddle-node bifurcation curve at (0.0079, −0.39), and runs to the upper right of the figure along with the saddle-node bifurcation curve SN2. As \( c_f \) increases, the contour lines of the periods diverge from each other and then vertically run to top of the figure. The stable periodic solution of the membrane potential is generated with the parameter values on the left side of the Hopf bifurcation curve. Figure 10 shows the typical waveform of the membrane potential corresponding to the stable periodic solution. A positive rightward shift of the Hopf bifurcation point by the increment of \( c_f \) indicates that similar to the case of \( I_{CaL} \) shown in Fig. 6, the range of \( c_{K1} \) for biological pacemaker generation is broadened by increasing \( I_f \).

4.3 Comparison of the additional current effects on the generation of the biological pacemaker activity

As shown respectively in Figs. 6, 8, 9, additional increments of \( I_{CaL} \), \( I_{NaCa} \), and \( I_f \) increase the maximum value of \( c_{K1} \) for pacemaker activity. Figure 11 shows the variation of the critical \( c_{K1} \) value for the pacemaker activity by changing \( I_{CaL} \), \( I_{NaCa} \), or \( I_f \). The \( c_{CaL} \) curve corresponds to the HB2 curve (in the range of \( 0 \leq c_{CaL} \leq 1.98 \)) and the SN2 curve (in the range of \( 1.98 < c_{CaL} \)) in Fig. 6. The \( c_{NaCa} \) curve is the same as the HB2 curve in Fig. 8, and the \( c_f \) curve is the HB2 curve in Fig. 9. Pacemaker activity appears below each curve. \( c_{NaCa} \) most effectively facilitates the generation of the pacemaker activity during \( I_{K1} \) suppression due to its wide parameter range for the pacemaker activity, but the difference between \( c_{CaL} \) and \( c_f \) is small. However, it should be noted that the scales of the x-axis are much larger than that of the y-axis. We did not take account of the physiologically feasibility of such large parameter values in the present study, which should be considered in the future.
Fig. 9. Two-parameter bifurcation diagram for $c_{K1}$ and $c_I$. (a) Global bifurcation structure. (b) Magnification of positive parameter range in Fig. 9(b). Red curve and blue curve designate the loci of the Hopf bifurcation point and the saddle-node bifurcation point in Fig. 2, respectively. Other black curves designate the loci of periodic solutions with specific periods. (Stable and unstable periodic solutions are denoted by solid curves and dashed curves, respectively.) Gray shaded area shows the parameter range for pacemaker activity.

Fig. 10. Simulated biological pacemaker activity as $c_I$ increases and $c_{K1}$ decreases. (a) $c_{K1} = 0.03, c_I = 3.0$. (b) $c_{K1} = 0.05, c_I = 6.0$.

Fig. 11. Comparison of the effects of $I_{CaL}, I_{NaCa}$, and $I_I$ on the critical value of $c_{K1}$ for the biological pacemaker activity.

5. Conclusions

We investigated an efficient and effective way to create a biological pacemaker from a human ventricular myocyte using the reduce TNNP model. We analyzed the bifurcation structure of the model by changing the conductances of all ion channels as bifurcation parameters, and searched the parameter regions for pacemaker activity. One-parameter bifurcation analyses for the conductances of single ion channels showed that spontaneous pacemaker activity appears by the suppression of $I_{K1}$. However, the parameter range of $c_{K1}$ for the pacemaker activity is extremely narrow. Therefore, we examined whether additional changes of other ionic currents facilitate biological pacemaker generation by $I_{K1}$ suppression. Thus, we conducted the two-parameter bifurcation analyses for almost all combinations
of two ion channel conductances. We found that the additional increment of \( I_{\text{CaL}} \), \( I_f \), or \( I_{\text{NaCa}} \) extends the \( c_{\text{K1}} \) range for the pacemaker activity and facilitates the generation of pacemaking. The suppression of \( I_{\text{K1}} \) is the simplest and most feasible method using current experimental technology. However, our findings on the coupling effects of two ionic currents provide a new perspective to experimental approaches for biological pacemaker engineering in the near future.

Although Kurata et al. [9, 17] showed a possible method for biological pacemaker creation using a mathematical model, model incompleteness due to the lack of experimental data from human cardiomyocytes limited their study. Furthermore, we present more detailed and comprehensive two-parameter bifurcation analyses, which includes the loci of periodic solutions with a specific period as well as bifurcation points.

In the present paper, we mainly focused on the induction of the pacemaker activity without evaluating its quantitative characteristics such as the period or amplitude, which are associated with the pacing rate and the driving ability of a biological pacemaker. We partially examined the validation of these characteristics, changing \( I_f \) with \( I_{\text{K1}} \) suppression [19]. The results showed that the action potential waveform of a biological pacemaker differs from that of the sinoatrial node in some respects. The biological pacemaker may function adequately as a pacemaker, even if its waveform slightly differs from the sinoatrial node. Therefore, we will consider the ventricular pacemaker ability to propagate the action potential to non-pacemaking cells using a coupled cell model as a future work.

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Appendix
A. Equations for the ionic currents of the reduced TNNP model
A.1 Equilibrium potentials
\[
E_X = \frac{RT}{z_X F} \log \frac{X_o}{X_i} \quad \text{for } X = \text{Na, K, Ca} \quad (A-1)
\]
\[
E_{\text{Ks}} = \frac{RT}{F} \log \frac{K_o + p_{\text{KNa}}Na_o}{K_i + p_{\text{KNa}}Na_i} \quad (A-2)
\]
A.2 Fast Na\(^{+}\) current
\[
I_{\text{Na}} = c_{\text{Na}}G_{\text{Na}}m^3h_j(V - E_{\text{Na}}) \quad (A-3)
\]
\[
m_\infty = \frac{1}{[1 + \exp((-56.86 - V)/9.03)]^2} \quad (A-4)
\]
\[
\alpha_m = \frac{1}{1 + \exp((-60 - V)/5)} \quad (A-5)
\]
\[
\beta_m = \frac{0.1}{1 + \exp((V + 35)/5)} + \frac{0.1}{1 + \exp((V - 50)/200)} \quad (A-6)
\]
\[
\tau_m = \alpha_m\beta_m \quad (A-7)
\]
\[
h_\infty = \frac{1}{[1 + \exp((V + 71.55)/7.43)]^2} \quad (A-8)
\]
\[
\alpha_h = \begin{cases} 
0 & (V \geq -40) \\
0.057\exp(-(V + 80)/6.8) & (V < -40)
\end{cases} \quad (A-9)
\]
\[
\beta_h = \begin{cases} 
0.13[1 + \exp(-(V + 10.66)/11.1)] & (V \geq -40) \\
2.7\exp(0.079V) + 3.1 \times 10^5\exp(0.3485V) & (V < -40)
\end{cases} \quad (A-10)
\]
\[
\tau_h = \frac{1}{\alpha_h + \beta_h} \quad (A-11)
\]
\[ j_\infty = \frac{1}{1 + \exp((V + 71.55)/7.43)^2} \]  
\[ \alpha_j = \begin{cases} 0 & (V \geq -40) \\ \left( -2.5428 \times 10^4 \exp(0.2444V) - 6.948 \times 10^{-6} \exp(-0.04391V) \right) (V + 37.78) & (V < -40) \end{cases} \]  
\[ \beta_j = \begin{cases} \frac{0.6 \exp(0.057V)}{1 + \exp(-0.1(V + 32))} & (V \geq -40) \\ \frac{0.02424 \exp(-0.01052V)}{1 + \exp(-0.1378(V + 40.14))} & (V < -40) \end{cases} \]  
\[ \tau_j = \frac{1}{\alpha_j + \beta_j} \]  
\[ A.3 \text{ L-type Ca}^{2+} \text{ current} \]

\[ I_{CaL} = c_{CaL} G_{CaL} d_\infty f_1 f_2 (V - 60) \]  
\[ d_\infty = \frac{1}{1 + \exp((-8 - V)/7.5)} \]  
\[ f_1 \infty = \frac{1}{1 + \exp((V + 20)/7)} \]  
\[ \tau_{f1} = 1102.5 \exp\left(-\frac{(V + 27)^2}{15}\right) + \frac{200}{1 + \exp((13 - V)/10)} + \frac{180}{1 + \exp((V + 30)/10)} + 20 \]  
\[ f_{2\infty} = \frac{0.67}{1 + \exp((V + 35)/7)} + 0.33 \]  
\[ \tau_{f2} = 600 \exp\left(-\frac{(V + 27)^2}{170}\right) + \frac{7.75}{1 + \exp((25 - V)/10)} + \frac{16}{1 + \exp((V + 30)/10)} \]  
\[ A.4 \text{ Transient outward current} \]

\[ I_{to} = c_{to} G_{to} r_\infty s(V - E_K) \]  
\[ r_\infty = \frac{1}{1 + \exp((20 - V)/6)} \]  
\[ s_\infty = \frac{1}{1 + \exp((V + 20)/5)} \]  
\[ \tau_s = 85 \exp\left(-\frac{(V + 45)^2}{320}\right) + \frac{5}{1 + \exp((V - 20)/5)} + 3 \]  
\[ A.5 \text{ Rapid delayed rectifier current} \]

\[ I_{Kr} = c_{Kr} G_{Kr} \sqrt{\frac{K_o}{5.4}} x_{r1} x_{r2\infty} (V - E_K) \]  
\[ x_{r1\infty} = \frac{1}{1 + \exp((-26 - V)/7)} \]  
\[ \tau_{xr1} = \frac{450}{1 + \exp((-45 - V)/10)} \cdot \frac{6}{1 + \exp((V + 30)/11.5)} \]  
\[ x_{r2\infty} = \frac{1}{1 + \exp((V + 88)/24)} \]  
\[ A.6 \text{ Slow delayed rectifier current} \]

\[ I_{Ks} = c_{Ks} G_{Ks} x_{s2}^2 (V - E_{Ks}) \]  
\[ x_{s\infty} = \frac{1}{1 + \exp((-5 - V)/14)} \]
| Parameter | Definition | Value |
|-----------|------------|-------|
| $R$ | Gas constant | $8.3143 \text{ JK}^{-1}\text{mol}^{-1}$ |
| $T$ | Temperature | $310 \text{ K}$ |
| $F$ | Faraday constant | $96.4867 \text{ C/mmole}$ |
| $z_{\text{Na}}$ | Valence of Na$^+$ | $1$ |
| $z_{\text{K}}$ | Valence of K$^+$ | $1$ |
| $z_{\text{Ca}}$ | Valence of Ca$^{2+}$ | $2$ |
| $N_{\text{a}}$ | Extracellular Na$^+$ concentration | $140 \text{ mM}$ |
| $K_{o}$ | Extracellular K$^+$ concentration | $5.4 \text{ mM}$ |
| $C_{\text{a}}$ | Extracellular Ca$^{2+}$ concentration | $2 \text{ mM}$ |
| $N_{\text{i}}$ | Intracellular Na$^+$ concentration | $7.67 \text{ mM}$ |
| $K_{i}$ | Intracellular K$^+$ concentration | $138.3 \text{ mM}$ |
| $C_{\text{i}}$ | Intracellular Ca$^{2+}$ concentration | $0.00007 \text{ mM}$ |
| $c_{\text{Na}}$ | Coefficient | $1.0$ |
| $G_{\text{Na}}$ | Maximal $I_{\text{Na}}$ conductance | $14.838 \text{ nS/pF}$ |
| $c_{\text{CaL}}$ | Coefficient | $1.0$ |
| $G_{\text{CaL}}$ | Maximal $I_{\text{CaL}}$ conductance | $0.2786 \text{ nS/pF}$ |
| $c_{\text{to}}$ | Coefficient | $1.0$ |
| $G_{\text{to}}$ | Maximal $I_{\text{to}}$ conductance | $0.294 \text{ nS/pF}$ |
| $c_{\text{Kr}}$ | Coefficient | $1.0$ |
| $G_{\text{Kr}}$ | Maximal $I_{\text{Kr}}$ conductance | $0.101 \text{ nS/pF}$ |
| $c_{\text{Ks}}$ | Coefficient | $1.0$ |
| $G_{\text{Ks}}$ | Maximal $I_{\text{Ks}}$ conductance | $0.257 \text{ nS/pF}$ |
| $p_{\text{KNa}}$ | Relative $I_{\text{Ks}}$ permeability to Na | $0.03$ |
| $c_{\text{K1}}$ | Coefficient | $1.0$ |
| $G_{\text{K1}}$ | Maximal $I_{\text{K1}}$ conductance | $5.405 \text{ nS/pF}$ |
| $c_{\text{NaCa}}$ | Coefficient | $1.0$ |
| $G_{\text{NaCa}}$ | Maximal $I_{\text{NaCa}}$ | $1000 \text{ pA/pF}$ |
| $\gamma$ | Voltage dependence parameter of $I_{\text{NaCa}}$ | $0.35$ |
| $K_{\text{mCa}}$ | Ca$^{2+}$ half-saturation constant for $I_{\text{NaCa}}$ | $1.38 \text{ mM}$ |
| $K_{\text{mNa}}$ | Na$^+$ half-saturation constant for $I_{\text{NaCa}}$ | $87.5 \text{ mM}$ |
| $h_{\text{sat}}$ | Saturation factor for $I_{\text{NaCa}}$ | $0.1$ |
| $\alpha$ | Factor enhancing the outward nature of $I_{\text{NaCa}}$ | $2.5$ |
| $c_{\text{NaK}}$ | Coefficient | $1.0$ |
| $G_{\text{NaK}}$ | Maximal $I_{\text{NaK}}$ | $2.724 \text{ pA/pF}$ |
| $K_{\text{mK}}$ | K$^+$ half-saturation constant of $I_{\text{NaK}}$ | $1 \text{ mM}$ |
| $K_{\text{mNa}}$ | Na$^+$ half-saturation constant of $I_{\text{NaK}}$ | $40 \text{ mM}$ |
| $c_{\text{pCa}}$ | Coefficient | $1.0$ |
| $G_{\text{pCa}}$ | Maximal $I_{\text{pCa}}$ conductance | $0.1238 \text{ nS/pF}$ |
| $K_{\text{pCa}}$ | Half-saturation constant of $I_{\text{pCa}}$ | $0.0005 \text{ mM}$ |
| $c_{\text{pK}}$ | Coefficient | $1.0$ |
| $G_{\text{pK}}$ | Maximal $I_{\text{pK}}$ conductance | $0.0293 \text{ nS/pF}$ |
| $G_{\text{bNa}}$ | Maximal $I_{\text{bNa}}$ conductance | $0.000290 \text{ nS/pF}$ |
| $G_{\text{bCa}}$ | Maximal $I_{\text{bCa}}$ conductance | $0.000592 \text{ nS/pF}$ |

$$\tau_{x_{x_1}} = \frac{1400}{\sqrt{1 + \exp((V - 35)/15)} + 80} \quad \text{(A-32)}$$

**A.7 Inward rectifier K$^+$ current**

$$I_{K1} = c_{K1}G_{K1}\sqrt{\frac{K_o}{5.4}}x_{K1\infty}(V - E_K) \quad \text{(A-33)}$$
Table B-I. Model parameters.

| Parameter | Definition | Value     |
|-----------|------------|-----------|
| $G_f$     | Maximum conductance | 0.075 nS/pF |
| $E_f$     | Equilibrium potential | $-22$ mV  |

\[
\alpha_{K1} = \frac{0.1}{1 + \exp(0.06(V - E_K - 200))}
\]
\[
\beta_{K1} = \frac{3\exp(0.0002(V - E_K + 100)) + \exp(0.1(V - E_K - 10))}{1 + \exp(-0.5(V - E_K))}
\]
\[
x_{K1\infty} = \frac{\alpha_{K1}}{\alpha_{K1} + \beta_{K1}}
\]

A.8 Na$^+$/Ca$^{2+}$ exchanger current

\[
I_{NaCa} = c_{NaCa} k_{NaCa} \frac{\exp(\gamma VF/RT)Na_i^3Ca_o - \exp((\gamma - 1)VF/RT)Na_o^3Ca_i \cdot \alpha}{(K_mNa_i + Na_o^3)(K_mC_a + Ca_o)(1 + k_{sat} \exp((\gamma - 1)VF/RT))}
\]

A.9 Na$^+$/K$^+$ pump current

\[
I_{NaK} = c_{NaK} P_{NaK} \frac{K_o Na_i}{(K_o + K_mK)(Na_i + K_mNa)(1 + 0.1245 \exp(-0.1VF/RT) + 0.0353 \exp(-VF/RT))}
\]

A.10 Plateau Ca$^{2+}$ current

\[
I_{pCa} = c_{pCa} G_{pCa} \frac{Ca_i}{K_{pCa} + Ca_i}
\]

A.11 Plateau K$^+$ current

\[
I_{pK} = c_{pK} G_{pK} \frac{V - E_K}{1 + \exp((25 - V)/5.98)}
\]

A.12 Background currents

\[
I_{bNa} = G_{bNa}(V - E_Na)
\]
\[
I_{bCa} = G_{bCa}(V - E_Ca)
\]

B. Equations for the hyperpolarization-activated current $I_f$ (Verkerk model)

\[
I_f = G_f y(V - E_f)
\]
\[
y_{\infty} = \frac{1}{1 + \exp(-(-96.9 - V)/8.8)}
\]
\[
\alpha_y = \frac{0.36(V + 148.8)}{\exp(0.066(V + 148.8)) - 1}
\]
\[
\beta_y = \frac{0.1(V + 87.3)}{1 - \exp(-0.21(V + 87.3))}
\]
\[
\tau_y = \frac{1}{(\alpha_y + \beta_y)} - 0.054
\]

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