A mathematical model of pacemaker activity recorded from mouse small intestine

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The pacemaker activity of interstitial cells of Cajal (ICCs) has been known to initiate the propagation of slow waves along the whole gastrointestinal tract through spontaneous and repetitive generation of action potentials. We studied the mechanism of the pacemaker activity of ICCs in the mouse small intestine and tested it using a mathematical model. The model includes ion channels, exchanger, pumps and intracellular machinery for Ca\(^{2+}\) regulation. The model also incorporates inositol 1,4,5-triphosphate (IP\(_3\)) production and IP\(_3\)-mediated Ca\(^{2+}\) release activities. Most of the parameters were obtained from the literature and were modified to fit the experimental results of ICCs from mouse small intestine. We were then able to compose a mathematical model that simulates the pacemaker activity of ICCs. The model generates pacemaker potentials regularly and repetitively as long as the simulation continues. The frequency was set at 20 min\(^{-1}\) and the duration at 50% repolarization was 639 ms. The resting and overshoot potentials were \(-78\) and \(+1.2\) mV, respectively. The reconstructed pacemaker potentials closely matched those obtained from animal experiments. The model supports the idea that cyclic changes in [Ca\(^{2+}\)]\(_i\) and [IP\(_3\)] play key roles in the generation of ICC pacemaker activity in the mouse small intestine.

Keywords: Cajal cell; pacemaker; small intestine; simulation

1. Introduction

Rhythmical contractions of the gastrointestinal (GI) tract are associated with the pacemaker electrical activity generated in the muscle layers, which occurs at...
low frequency in the absence of an extrinsic nervous stimulation. The ICCs, distributed in the myenteric region of the GI wall, have been suggested to initiate the pacemaker activity (Tomita 1981), which then propagates to the smooth muscle cells through gap junctions to generate the contraction of the whole GI tract (Dickens et al. 1999). This pacemaker activity is represented by the driving potential or pacemaker potential of ICC. However, the mechanism of the pacemaker activity is not yet well understood.

Recently, Goto et al. (2004) recorded the pacemaker activity in a single ICC prepared from mouse small intestine. The amplitude of the pacemaker potential was slightly above 70 mV and a series of voltage-clamp experiments was performed to investigate the mechanism of the pacemaker activity. They recorded a large inward current with an autonomous time course ($I_{\text{AI}}$) by applying a depolarizing pulse and concluded that the spontaneous depolarization is caused by the activation of the $I_{\text{AI}}$. Now, it is possible to develop a mathematical model to explain the regenerative nature of pacemaker potentials in a single ICC. Although some investigators have made mathematical models to simulate the regenerative potentials in the GI tract (Miftakhov et al. 1999; Edwards & Hirst 2003, 2005), the models are still phenomenological in the point of method or approach, and they focused on the electrical activity of a larger cellular network rather than a single ICC.

In this study, pacemaker potentials of ICCs from the mouse small intestine were simulated with a mathematical model to explain the regenerative nature of the pacemaker potentials and the underlying $[\text{Ca}^{2+}]_i$ changes. The model faithfully reproduces the pacemaker activity and suggests a possible mechanism.

2. Methods

Modelling of the pacemaker activity in the ICCs is still at a very early stage in comparison with modelling of the pacemaker activity of the heart. Furthermore, the experimental results describing the $\text{Ca}^{2+}$ dynamics, ion channels and intracellular metabolic pathways in ICCs are scarce. Thus, although our object is to make a simulation model of mouse ICCs, the equations and parameters used were partly derived from the heart models (Luo & Rudy 1994; Matsuoka et al. 2003) and were modified to reproduce the pacemaker activity of mouse ICCs (table 1).

(a) Cellular geometry

The ICCs isolated from mouse small intestine have a spindle-shaped cell body with extending processes. The cell body is 5–15 µm wide, and the measured mean capacitance is 21–25 pF (Kim et al. 2002; Koh et al. 2002; Goto et al. 2004). The cell volume is simply calculated from a cell capacitance of 25 pF by assuming that the cell has the same hexahedral geometry as guinea-pig ventricular myocytes ($100 \times 20 \times 8 \; \mu\text{m}^3$) except for its smaller scale in cell dimension (see Matsuoka et al. 2003). Given the surface area provided by the capacitance and the hexahedral geometry, the cell dimension was determined and used to calculate the cell volume. The $V_i$ for ion diffusion was assumed to be 50% of the cell volume (Matsuoka et al. 2003). We also divided the SR into a $\text{Ca}^{2+}$-release
site in the junctional space and a Ca\(^{2+}\)-uptake site in the deep cytoplasmic space (Mackenzie et al. 2001). The cell capacitance and the volume of each compartment are summarized in table 2.

(b) Ca\(^{2+}\)-binding proteins

The amounts of Ca\(^{2+}\) that bound to troponin, calmodulin and calsequestrin were estimated using the approach described by Luo & Rudy (1994). We assumed that the rates of Ca\(^{2+}\) binding are so fast that the concentration of free calcium gets equilibrium with the Ca\(^{2+}\)-binding proteins instantaneously. The concentrations of free calcium and Ca\(^{2+}\)-binding proteins satisfy the following

Table 1. Abbreviations and symbols.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICCs</td>
<td>interstitial cells of Cajal</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>(C_m)</td>
<td>membrane capacitance, pF</td>
</tr>
<tr>
<td>(V)</td>
<td>membrane potential, mV</td>
</tr>
<tr>
<td>(V_i)</td>
<td>cell volume accessible for ion diffusion, (\mu)m(^3)</td>
</tr>
<tr>
<td>(V_{up})</td>
<td>volume of SR uptake site, (\mu)m(^3)</td>
</tr>
<tr>
<td>(V_{rel})</td>
<td>volume of SR release site, (\mu)m(^3)</td>
</tr>
<tr>
<td>(I_o)</td>
<td>ion X component of current (I_o), pA</td>
</tr>
<tr>
<td>(I_{net,X})</td>
<td>whole cell current carried by ion X, pA</td>
</tr>
<tr>
<td>(I_{ext})</td>
<td>current applied through the electrode, pA</td>
</tr>
<tr>
<td>(I_{tot})</td>
<td>total current of ion channels, exchanger and pumps, pA</td>
</tr>
<tr>
<td>(X_{CF})</td>
<td>constant field equation for ion (X), mM</td>
</tr>
<tr>
<td>(E_X)</td>
<td>equilibrium potential for ion (X), mV</td>
</tr>
<tr>
<td>(F)</td>
<td>Faraday constant, 96.4867 C mmol(^{-1})</td>
</tr>
<tr>
<td>(R)</td>
<td>gas constant, 8.3143 C mVK(^{-1}) mmol(^{-1})</td>
</tr>
<tr>
<td>(T)</td>
<td>absolute temperature, K</td>
</tr>
<tr>
<td>(z_X)</td>
<td>valence of ion (X)</td>
</tr>
<tr>
<td>([X]_i)</td>
<td>intracellular concentration of ion (X), mM</td>
</tr>
<tr>
<td>([X]_o)</td>
<td>extracellular concentration of ion (X), mM</td>
</tr>
<tr>
<td>([X]_f)</td>
<td>concentration of (X) in the free form, mM</td>
</tr>
<tr>
<td>([X]_{total})</td>
<td>concentration of (X) in the free and bound form, mM</td>
</tr>
<tr>
<td>([Ca]_i)</td>
<td>total cytosolic calcium concentration in the free and bound form in the release pool of SR, mM</td>
</tr>
<tr>
<td>TRPN</td>
<td>troponin</td>
</tr>
<tr>
<td>CMDN</td>
<td>calmodulin</td>
</tr>
<tr>
<td>CSQN</td>
<td>calsequestrin</td>
</tr>
<tr>
<td>PIP(_2)</td>
<td>phosphatidylyl-1,4-bisphosphate</td>
</tr>
<tr>
<td>IP(_3)</td>
<td>inositol 1,4,5-triphosphate</td>
</tr>
<tr>
<td>IP(_4)</td>
<td>inositol 1,3,4,5-tetraphosphate</td>
</tr>
<tr>
<td>IP(_3)R</td>
<td>inositol 1,4,5-triphosphate receptor channel</td>
</tr>
<tr>
<td>(Km_X)</td>
<td>Michaelis constant for ion (X) binding</td>
</tr>
<tr>
<td>C, O, I, B</td>
<td>closed, open, inactivated and blocked state, respectively</td>
</tr>
<tr>
<td>(p_o)</td>
<td>open probability of a channel</td>
</tr>
<tr>
<td>(m, h)</td>
<td>activation and inactivation gate, respectively</td>
</tr>
<tr>
<td>(\alpha_m, \beta_m)</td>
<td>opening and closing rate constants, respectively, of (m), ms(^{-1})</td>
</tr>
<tr>
<td>(\alpha_h, \beta_h)</td>
<td>opening and closing rate constants, respectively, of (h), ms(^{-1})</td>
</tr>
</tbody>
</table>

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Table 2. Cell geometry.

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_m</td>
<td>25 pF</td>
</tr>
<tr>
<td>V_i</td>
<td>715.5 μm³</td>
</tr>
<tr>
<td>V_up</td>
<td>0.1 V_i</td>
</tr>
<tr>
<td>V_rel</td>
<td>0.01 V_i</td>
</tr>
</tbody>
</table>

Table 3. Ca²⁺-binding proteins.

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km_TRPN</td>
<td>0.0005 mM</td>
</tr>
<tr>
<td>Km_CMDN</td>
<td>0.00238 mM</td>
</tr>
<tr>
<td>Km_CSQN</td>
<td>0.8 mM</td>
</tr>
</tbody>
</table>

equations at equilibrium (table 3):

\[
[\text{Ca}^{2+}]_i = \frac{[\text{Ca}]_i}{1 + ([\text{TRPN}]_f/Km_{\text{TRPN}}) + ([\text{CMDN}]_f/Km_{\text{CMDN}})}, \tag{2.1}
\]

\[
[\text{TRPN}]_f = \frac{[\text{TRPN}]_{\text{total}}}{1 + ([\text{Ca}^{2+}]_f/Km_{\text{TRPN}})}, \tag{2.2}
\]

\[
[\text{CMDN}]_f = \frac{[\text{CMDN}]_{\text{total}}}{1 + ([\text{Ca}^{2+}]_f/Km_{\text{CMDN}})}, \tag{2.3}
\]

\[
[\text{Ca}^{2+}]_{\text{rel}} = \frac{[\text{Ca}]_{\text{rel}}}{1 + ([\text{CSQN}]_f/Km_{\text{CSQN}})}, \tag{2.4}
\]

\[
[\text{CSQN}]_f = \frac{[\text{CSQN}]_{\text{total}}}{1 + ([\text{Ca}^{2+}]_{\text{rel}}/Km_{\text{CSQN}})}. \tag{2.5}
\]

(c) Calculation of the membrane potential and internal ion concentrations

(i) Membrane potential

Time-dependent changes in the membrane potential are described by the following equation:

\[
dV/dt = -(I_{\text{tot}} + I_{\text{ext}})/C_m, \tag{2.6}
\]

where \(I_{\text{ext}}\) is the current applied through the electrode by the current clamp or whole-cell voltage-clamp circuitry. \(I_{\text{tot}}\) includes an inward rectifier K⁺ current (\(I_K\)), an L-type Ca²⁺ current (\(I_{\text{CaL}}\)), a voltage-dependent and dihydropyridine

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(DHP)-resistant current ($I_{VDDR}$), an autonomous inward current ($I_{AI}$), a Na$^+$/Ca$^{2+}$ exchange current ($I_{NaCa}$), a Na$^+$/K$^+$ pump current ($I_{NaK}$) and a plasmalemmal Ca$^{2+}$ pump current ($I_{PMCA}$):

$$I_{tot} = I_{K1} + I_{CaL} + I_{VDDR} + I_{AI} + I_{NaCa} + I_{NaK} + I_{PMCA}. \quad (2.7)$$

(ii) Internal ion concentration

The cytosolic ionic concentrations were determined by the net ion fluxes across the plasma and SR membranes. The net ion fluxes were separated into three ionic components (Na$^+$, K$^+$ and Ca$^{2+}$) based on the ionic selectivity of each ion channels, exchanger and pumps. The ionic component of each channel current is described by the constant field equation (see equation (2.14)).

$$\frac{d}[Na^+]_i}{dt} = -I_{netNa}/(z_Na F V_i), \quad (2.8)$$

$$\frac{d}[K^+]_i}{dt} = -I_{netK}/(z_K F V_i), \quad (2.9)$$

$$\frac{d}[Ca^{2+}]_i}{dt} = -(I_{netCa} - I_{IP3R} + I_{up} - I_{leak})/(z_Ca F V_i), \quad (2.10)$$

$$I_{netNa} = I_{CaL} Na + I_{AI} Na + 3I_{NaK} + 3I_{NaCa}, \quad (2.11)$$

$$I_{netK} = I_{K1} + I_{CaL} K + I_{AI} K - 2I_{NaK}, \quad (2.12)$$

$$I_{netCa} = I_{CaL} Ca + I_{VDDR} + I_{AI} Ca - 2I_{NaCa} + I_{PMCA}, \quad (2.13)$$

$$X_{CF} = \frac{z_X F V}{RT} \left[ [X^X]_i - [X^X]_0 \exp\left(-\frac{z_X F V}{RT} \right) \right] \frac{\exp\left(-\frac{z_X F V}{RT} \right)}{1 - \exp\left(-\frac{z_X F V}{RT} \right)}. \quad (2.14)$$

(d) Ion channels

(i) Inward rectifier $K^+$ current ($I_{K1}$)

The $I_{K1}$ has been reported in cultured ICCs from the mouse small intestine (Koh et al. 1998). The $I_{K1}$ is thought to repolarize the pacemaker potential. We adopted the Kyoto model (Matsuoka et al. 2003) and modified it to reproduce the pacemaker activity of ICCs, as described below (table 4):

State C goes into state O reversibly,

$$C \xrightarrow{\alpha_a}{\beta_a} O.$$ 

State O also goes into state B reversibly,

$$O \xrightarrow{\mu}{\lambda} B.$$
We assumed the transition between state O and B occurs instantaneously.

\[ I_{K1} = G_{K1} ([K^+]_o/5.4)^{0.62} (V - E_K) n f_U, \] (2.15)

\[ \frac{\alpha_n}{\alpha_n + \beta_n} = \frac{1}{1 + 0.1 \exp((V - E_K)/15) + 0.048 \exp((V - E_K)/7)}, \] (2.16)

\[ \alpha_n + \beta_n = \frac{2.5}{4.7 \exp((V - E_K)/28.7)} + \frac{2.5}{6.0 \exp((V - E_K)/25.8)}, \] (2.17)

\[ \mu = 3.3 \exp((V - E_K - 6)/15), \] (2.18)

\[ \lambda = 26 \exp((E_K - V - 6)/52), \] (2.19)

\[ f_B = \frac{\mu}{\mu + \lambda}, \] (2.20)

\[ f_U = \frac{\lambda}{\mu + \lambda}. \] (2.21)

(ii) L-type \( Ca^{2+} \) current \( (I_{CaL}) \)

The depolarization of an ICC is thought to activate the \( I_{CaL} \) in the neighbouring ICC (Kim et al. 2002). The presence of an L-type \( Ca^{2+} \) channel was clearly demonstrated by Cho & Daniel (2005) using the double-immunofluorescence labelling method. We adopted the classical two-state model and used the rate constants obtained from the voltage-clamp data of Kim et al. (2002). In addition, we added the \( Ca^{2+} \)-dependent inactivation kinetics based on the Kyoto model (Matsuoka et al. 2003).

\[ I_{CaL} = I_{CaL}Ca + I_{CaL}K + I_{CaL}Na, \] (2.22)

\[ I_{CaL}Ca = P_{CaL}Ca_{CF} mh, \] (2.23)

\[ I_{CaL}K = 0.001 P_{CaL}K_{CF} mh, \] (2.24)
\( I_{\text{CaLNa}} = 0.00005P_{\text{CaLNaCF}} mh, \) \hspace{1cm} (2.25)

\[
\alpha_m = 0.002175 \frac{V + 30}{1 - \exp((V + 30)/(-2.5))},
\]

\( \beta_m = 0.0006315 \frac{V}{\exp(V/2.5) - 1}, \) \hspace{1cm} (2.27)

\[
\alpha_h = 1.775 \times 10^{-6} \frac{V + 34}{\exp((V + 34)/5.633) - 1},
\]

\[
\beta_h = 0.427[\text{Ca}^{2+}]_i \frac{V + 64}{\exp((V + 44)/(-4.16)) + 1}.
\] \hspace{1cm} (2.29)

(iii) **Voltage-dependent and DHP-resistant current \( (I_{\text{VDDR}}) \)**

The \( I_{\text{VDDR}} \) is thought to contribute to slow wave propagation in the GI tract. The \( I_{\text{VDDR}} \) was fully studied and described by *Kim et al.* (2002) in the ICCs isolated from mouse colon and small intestine. We used the parameter values obtained from their results to reconstruct the \( I_{\text{VDDR}} \):

\[
I_{\text{VDDR}} = P_{\text{VDDR}} C_{\text{aCF}} mh,
\]

\[
\frac{\alpha_m}{\alpha_m + \beta_m} = \frac{1}{1 + \exp((V + 26)/(-6))},
\] \hspace{1cm} (2.31)

\[
\frac{1}{\alpha_m + \beta_m} = 0.6 + \frac{5.4}{1 + \exp(0.03(V + 100))},
\] \hspace{1cm} (2.32)

\[
\frac{\alpha_h}{\alpha_h + \beta_h} = \frac{1}{1 + \exp((V + 66)/6)},
\] \hspace{1cm} (2.33)

\[
\frac{1}{\alpha_h + \beta_h} = 10 + \frac{400}{1 + \exp(0.02(V + 65))},
\] \hspace{1cm} (2.34)

(iv) **Autonomous inward current \( (I_{\text{AI}}) \)**

*Goto et al.* (2004) identified a large transient inward current evoked by depolarization under voltage-clamp conditions (see figure 1). They named the current as the autonomous inward current \( (I_{\text{AI}}) \) in that it shows an inward current with an autonomous time course by depolarizing clamp pulses. After \( I_{\text{AI}} \) was triggered, it took a regenerative time course and lasted about 500 ms. The reversal potential was around +3 mV suggesting that \( I_{\text{AI}} \) is a non-selective cation current. These authors proposed the \( I_{\text{AI}} \) as the pacemaker current generating the spontaneous depolarization of ICCs without an electrical

stimulus. We added a \([\text{Ca}^{2+}]_i\)-dependent activation (see equation (2.39)) to reproduce a current with an autonomous time course.

\[
I_{\text{AI}} = I_{\text{AI}}^{\text{Ca}} + I_{\text{AI}}^{\text{K}} + I_{\text{AI}}^{\text{Na}},
\]

\[
I_{\text{AI}}^{\text{Ca}} = P_{\text{AI}}^{\text{Ca}} C_{\text{p}} \theta,
\]

\[
I_{\text{AI}}^{\text{K}} = 0.36 P_{\text{AI}}^{\text{K}} C_{\text{p}} \theta,
\]

\[
I_{\text{AI}}^{\text{Na}} = 0.4 P_{\text{AI}}^{\text{Na}} C_{\text{p}} \theta,
\]

\[
p_{\theta} = 0.0017 + \frac{(1 - 0.0017)}{1 + (K_{\text{mCai}}/[\text{Ca}^{2+}]_i)}.
\]

(e) **Exchanger and pumps**

(i) \(\text{Na}^+ / \text{Ca}^{2+}\) exchange current \((I_{\text{NaCa}})\)

The kinetic model and scheme are basically identical to those of the Kyoto model (Matsuoka et al. 2003). The six-state model of \(\text{Na}^+ / \text{Ca}^{2+}\) exchange was lumped into a two-state model according to Powell et al. (1993). The Michaelis constant \((K_m)\) and conversion factor \((P_{\text{NaCa}})\) were adjusted empirically to reproduce the repetitive and stable firing of pacemaker potentials (see figure 1; table 5)

\[
E_2\text{Na} \leftrightarrow K_{\text{mNa}} \leftrightarrow E_2 \leftrightarrow K_{\text{mCa}} \leftrightarrow E_2\text{Ca} \quad (1 - y)
\]

\[
k_1 \uparrow \downarrow k_2 \quad k_3 \uparrow \downarrow k_4 \quad \beta_y \uparrow \downarrow \alpha_y
\]

\[
E_1\text{Na} \leftrightarrow K_{\text{mNa}} \leftrightarrow E_1 \leftrightarrow K_{\text{mCa}} \leftrightarrow E_1\text{Ca} \quad y
\]

Figure 1. Reconstruction of pacemaker potentials in the ICCs from mouse small intestine. The simulation model fires regularly at a frequency of 20 min\(^{-1}\). The diastolic time-interval between two successive pacemaker potentials (from 90% repolarization of the previous pacemaker potential to 10% depolarization of the next pacemaker potential) was approximately 1840 ms. This pacemaker activity was so stable that there was no significant changes in the electrical property after running the simulation for a period corresponding to 10 min on a cellular time-scale.
\[ I_{\text{NaCa}} = P_{\text{NaCa}}(k_1 p(E_1 \text{Na}) y - k_2 p(E_2 \text{Na})(1 - y)), \]
\[ k_1 = \exp(0.32 F V / (R T)), \]
\[ k_2 = \exp((0.32 - 1) F V / (R T)), \]
\[ k_3 = 1, \]
\[ k_4 = 1, \]
\[ p(E_1 \text{Na}) = \frac{1}{1 + ((1 + [\text{Ca}^{2+}]_i / K_{\text{Ca}}) / ([\text{Na}^{+}]_i / K_{\text{Na}}))}, \]
\[ p(E_2 \text{Na}) = \frac{1}{1 + ((1 + [\text{Ca}^{2+}]_i / K_{\text{Ca}}) / ([\text{Na}^{+}]_o / K_{\text{Na}}))}, \]
\[ p(E_1 \text{Ca}) = \frac{1}{1 + ((1 + [\text{Na}^{+}]_i / K_{\text{Na}}) / ([\text{Ca}^{2+}]_i / K_{\text{Ca}}))}, \]
\[ p(E_2 \text{Ca}) = \frac{1}{1 + ((1 + [\text{Na}^{+}]_o / K_{\text{Na}}) / ([\text{Ca}^{2+}]_o / K_{\text{Ca}}))}, \]
\[ \alpha_y = k_2 p(E_2 \text{Na}) + k_4 p(E_2 \text{Ca}), \]
\[ \beta_y = k_1 p(E_1 \text{Na}) + k_3 p(E_1 \text{Ca}). \]

(ii) \( \text{Na}^+ / \text{K}^+ \) pump current \( I_{\text{NaK}} \)

Electrogenic \( \text{Na}^+ / \text{K}^+ \) pump extrudes three \( \text{Na}^+ \) ions in exchange for two \( \text{K}^+ \) ions generating a net outward current. As the kinetics of the \( \text{Na}^+ / \text{K}^+ \) pump have not been studied well in ICCs, we employed the model of Sakai \textit{et al.} (1996)
obtained from rabbit sino-atrial node cells. The $P_{\text{NaK}}$ was adjusted to maintain the $[\text{Na}^+]_i$ below 20 mM against the large $\text{Na}^+$ influx by $I_{\text{AI}}$ during the depolarizing phase of a pacemaker potential. The Michaelis constant ($K_m$) was also adjusted to fit the experimental results.

\[
I_{\text{NaK}} = \frac{P_{\text{NaK}}}{1 + (K_{m_{\text{Na}}} / [\text{Na}^+]_i)^{1.36}} \frac{1 - (V + 50)/250^2}{1 + (K_{m_{\text{Ko}}} / [\text{K}^+]_o)^2}. \tag{2.51}
\]

(iii) **Plasmalemmal Ca$^{2+}$ pump current ($I_{\text{PMCA}}$)**

The plasmalemmal Ca$^{2+}$ pump was clearly demonstrated in the ICCs by Cho & Daniel (2005) using the double-immunofluorescence labelling technique. However, the kinetic properties of the pump are not yet well understood. For this reason, we employed a general approach for the modelling of Ca$^{2+}$ pumps (Fridlyand et al. 2003). The half-activation calcium concentration was set to 8 $\mu$M to prevent an excessive Ca$^{2+}$ rise in the cytosolic space of ICCs.

\[
I_{\text{PMCA}} = \frac{P_{\text{PMCA}}}{1 + (K_{m_{\text{Cai}}} / [\text{Ca}^{2+}]_i)^2}. \tag{2.52}
\]

(f) **IP$_3$ receptor channel and SR Ca$^{2+}$ dynamics**

(i) **SR Ca$^{2+}$ pump current ($I_{\text{up}}$)**

The Ca$^{2+}$ handling by the SR Ca$^{2+}$ pump has been suggested to play a key role in the regulation of Ca$^{2+}$-dependent pacemaker currents, which were proposed to make a pacemaker depolarization (Hirst et al. 2002; Goto et al. 2004). The $I_{\text{up}}$ was calculated according to the model of Hilgemann & Noble (1987). The basic scheme is identical to that of the Kyoto model (Matsuoka et al. 2003) with a slight modification to the $K_m$ values and conversion factor (tables 6 and 7).

\[
E_2 \text{Ca} \xleftrightarrow[K_m_{\text{Cai}}]{k_1 \uparrow \downarrow} E_2 \quad (1 - y)
\]
\[
k_1 \uparrow \downarrow k_2 \quad k_3 \uparrow \downarrow k_4 \quad \beta_y \uparrow \downarrow \alpha_y
\]
\[
E_1 \text{Ca} \xrightarrow[K_m_{\text{Cai}}]{k_3 \uparrow \downarrow} E_1 \quad y
\]

\[
I_{\text{up}} = P_{\text{up}} (k_2 p(E_2 \text{Ca})(1 - y) - k_1 p(E_1 \text{Ca})y), \tag{2.53}
\]

\[
k_1 = 0.01, \tag{2.54}
\]

\[
k_2 = 1, \tag{2.55}
\]

\[
k_3 = 1, \tag{2.56}
\]

\[
k_4 = 0.01, \tag{2.57}
\]

Table 6. SR Ca\(^{2+}\) dynamics.

<table>
<thead>
<tr>
<th>(I_{\text{up}})</th>
<th>(P_{\text{up}}): conversion factor, 7728</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p(X)): probability of state X</td>
<td></td>
</tr>
<tr>
<td>(K_{\text{mCa}i}): 0.08 mM</td>
<td></td>
</tr>
<tr>
<td>(K_{\text{mCa}o}): 0.002 mM</td>
<td></td>
</tr>
<tr>
<td>(y): gate in the reduced two-state model</td>
<td></td>
</tr>
<tr>
<td>(\alpha_y), (\beta_y): forward and backward rate constants, respectively, of (y), ms(^{-1})</td>
<td></td>
</tr>
<tr>
<td>(I_{\text{tr}})</td>
<td>(P_{\text{tr}}): conversion factor, 20.5 pA mM(^{-1})</td>
</tr>
<tr>
<td>(I_{\text{leak}})</td>
<td>(P_{\text{leak}}): conversion factor, 0.45 pA mM(^{-1})</td>
</tr>
<tr>
<td>(I_{\text{IP3R}})</td>
<td>(P_{\text{IP3R}}): conversion factor, 217.2 pA mM(^{-1})</td>
</tr>
<tr>
<td>(K_{\text{mCa}i}): 0.01 (\mu)M</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Initial values.

| \([\text{Ca}^{2+}]_i\) | cytosolic \(\text{Ca}^{2+}\) concentration, 38 nM |
| \([\text{Ca}^{2+}]_{\text{rel}}\) | \(\text{Ca}^{2+}\) concentration in the release pool of SR, 4.6 mM |
| \([\text{Ca}^{2+}]_{\text{up}}\) | \(\text{Ca}^{2+}\) concentration in the uptake pool of SR, 13.1 mM |
| \([\text{IP}_3]\) | \(\text{IP}_3\) concentration, 0.268 \(\mu\)M |
| \([\text{PIP}_2]\) | \(\text{PIP}_2\) concentration, 2.66 \(\mu\)M |
| \([\text{IP}_4]\) | \(\text{IP}_4\) concentration, 0.0067 \(\mu\)M |
| \([\text{PI}_{\text{total}}]\) | total inositol phosphates concentration, 3.3 \(\mu\)M |
| \([K^+]_i\) | intracellular \(K^+\) concentration, 140 mM |
| \([K^+]_o\) | extracellular \(K^+\) concentration, 5.4 mM |
| \([\text{Na}^+]_i\) | intracellular \(\text{Na}^+\) concentration, 5.4 mM |
| \([\text{Na}^+]_o\) | extracellular \(\text{Na}^+\) concentration, 140 mM |

\[
p(E_1 \text{Ca}) = \frac{1}{1 + K_{\text{mCa}i}/[\text{Ca}^{2+}]_{\text{up}}}, \quad (2.58)
\]

\[
p(E_2 \text{Ca}) = \frac{1}{1 + K_{\text{mCa}o}/[\text{Ca}^{2+}]_i}, \quad (2.59)
\]

\[
p(E_1) = \frac{1}{1 + [\text{Ca}^{2+}]_{\text{up}}/K_{\text{mCa}i}}, \quad (2.60)
\]

\[
p(E_2) = \frac{1}{1 + [\text{Ca}^{2+}]_i/K_{\text{mCa}o}}, \quad (2.61)
\]

\[
\alpha_y = k_2 p(E_2 \text{Ca}) + k_4 p(E_2), \quad (2.62)
\]

\[
\beta_y = k_1 p(E_1 \text{Ca}) + k_3 p(E_1). \quad (2.63)
\]
(ii) \( \text{Ca}^{2+} \) transfer from the SR uptake site to the release site \((I_{\text{tr}})\)

In the cardiac myocyte modelling, the SR \( \text{Ca}^{2+} \) pool was divided into the uptake and release sites to describe the underlying basis for the mechanical restitution and force–frequency relationship (Luo & Rudy 1994; Matsuoka et al. 2003). There is also evidence that a force–frequency relationship exists in the gastric smooth muscle cells, a similar cell type to ICC (Fukuta et al. 2002). Therefore, we separated the SR \( \text{Ca}^{2+} \) pool of ICC into two different regions. The \( \text{Ca}^{2+} \) transfer is suggested to move \( \text{Ca}^{2+} \) from the uptake site to the release site of the SR to provide \( \text{Ca}^{2+} \) for the next release. We used the scheme identical to that of the Kyoto model and adjusted the conversion or amplitude factor \((P_{\text{tr}})\) to fit the time course of the autonomous inward current (see figure 3) representing the cytosolic \( \text{Ca}^{2+} \) transient.

\[
I_{\text{tr}} = P_{\text{tr}}([\text{Ca}^{2+}]_{\text{up}} - [\text{Ca}^{2+}]_{\text{rel}}). \tag{2.64}
\]

(iii) \( \text{Ca}^{2+} \) leak from the SR \((I_{\text{leak}})\)

The \( I_{\text{leak}} \) was also taken from a scheme identical to that of the Kyoto model, and the conversion factor \((P_{\text{leak}})\) was adjusted to fit the time course of the cytosolic \( \text{Ca}^{2+} \) transient.

\[
I_{\text{leak}} = P_{\text{leak}}([\text{Ca}^{2+}]_{\text{up}} - [\text{Ca}^{2+}]_{i}). \tag{2.65}
\]

(iv) \( \text{IP}_3 \)-mediated \( \text{Ca}^{2+} \) release from the SR \((I_{\text{IP3R}})\)

The \( \text{IP}_3 \)-mediated \( \text{Ca}^{2+} \) release has been suggested to mediate the generation of pacemaker potential via the activation of a \( \text{Ca}^{2+} \)-dependent inward current (Ward et al. 2000; Malysz et al. 2001; Goto et al. 2004). There is an increasing body of evidence that the \( I_{\text{IP3R}} \) in the SR is dependent on both the \( \text{Ca}^{2+} \) and \( \text{IP}_3 \) concentrations (Bezprozvanny et al. 1991; Iino et al. 1993; Marchant & Taylor 1997; Taylor & Laude 2002). We employed the model of Marchant & Taylor (1997), which describes the opening of \( \text{IP}_3 \) receptors by sequential binding of \( \text{IP}_3 \) and \( \text{Ca}^{2+} \). In their scheme, the binding of \( \text{IP}_3 \) rapidly changes the conformation of the receptor to expose a \( \text{Ca}^{2+} \)-binding site; \( \text{Ca}^{2+} \) then binds to this newly exposed site, and the channel opens allowing \( \text{Ca}^{2+} \) to pass through. They proposed that three or four subunits of the \( \text{IP}_3 \) receptor must bind \( \text{IP}_3 \) before the channel opens and releases \( \text{Ca}^{2+} \) into the cytosol. We added another term, \( \text{Ca}^{2+} \) concentration at the release site of the SR \(([\text{Ca}^{2+}]_{\text{rel}})\), to allow the rate of \( \text{Ca}^{2+} \) release to be dependent on the fluctuation of the SR \( \text{Ca}^{2+} \) pool (see equation (2.67)). All the rate constants were modified to reproduce the \( I_{\text{AI}} \).

\[
\begin{align*}
C & \xrightarrow{k_o} O \\
k_u & \xleftrightarrow{k_i} \downarrow k_a \\
I_{\text{inactivated state}} \\
I_{\text{IP3R}} &= P_{\text{IP3R}}([\text{Ca}^{2+}]_{\text{rel}} - [\text{Ca}^{2+}]_{i})p_o, \tag{2.66} \\
k_{ic} &= 0.0025([\text{IP}_3]/0.001)^3[\text{Ca}^{2+}]_{\text{rel}}, \tag{2.67}
\end{align*}
\]
Mathematical model of pacemaker activity

\[ k_{ci} = 0.000849, \]  
\[ k_{co} = 0.147([\text{Ca}^{2+}]_i/0.000057)^3, \]  
\[ k_{oi} = 0.02. \]  

(v) \textit{Ca}^{2+} concentration in the SR

The \textit{Ca}^{2+} concentrations of the SR uptake and release sites were calculated using the following equations:

\[
d[\text{Ca}^{2+}]_{\text{up}}/dt = (I_{\text{up}} - I_{\text{tr}} - I_{\text{leak}})/(z_{\text{Ca}} V_{\text{up}} F), \tag{2.71}
\]
\[
d[\text{Ca}^{2+}]_{\text{rel}}/dt = (I_{\text{tr}} - I_{\text{IP3R}})/(z_{\text{Ca}} V_{\text{rel}} F). \tag{2.72}
\]

(g) \textit{IP}_3 metabolism

The \textit{IP}_3 plays a central role in mobilizing \textit{Ca}^{2+} in eukaryotic cells (Berridge & Irvine 1989; Rana & Hokin 1990). The binding of hormone to the receptors on a cell surface activates the phospholipase C (PLC), which subsequently hydrolyses PIP$_2$ in the plasma membrane into the \textit{IP}_3 and diacylglycerol. The \textit{IP}_3 finally opens a channel on the SR to release the stored \textit{Ca}^{2+} into the cytosolic space (\textit{IP}_3-mediated \textit{Ca}^{2+} release) and \textit{IP}_3 is then recycled to the plasma membrane after its degradation to the inactive forms. There is an increasing body of evidence that \textit{IP}_3 formation is also dependent on the membrane depolarization (Vergara et al. 1985; Best & Bolton 1986; Wang et al. 1995; Ganitkevich & Isenberg 1996; Goto et al. 2004). We created a kinetic scheme to reproduce the depolarization-evoked rise of \textit{IP}_3-mediated \textit{Ca}^{2+} release in the ICCs from mouse small intestine (Goto et al. 2004). The following scheme simplifies the metabolic pathways of inositol phosphates (PIP$_2$, \textit{IP}_3, \textit{IP}_4 and other metabolites):

\[
\begin{aligned}
\text{PIP}_2 &\xrightarrow{k_{ca}} \text{IP}_3 \\
\text{IP}_4 &\xrightarrow{k_{bc}} \text{IP}_3
\end{aligned}
\]

The time-dependent changes of inositol phosphates can be described by the following equations:

\[
d[\text{IP}_3]/dt = k_{ab}[\text{PIP}_2] - (k_{ba} + k_{bc})[\text{IP}_3], \tag{2.73}
\]
\[
d[\text{PIP}_2]/dt = k_{ba}[\text{IP}_3] + k_{ca}[\text{IP}_4] - k_{ab}[\text{PIP}_2], \tag{2.74}
\]
\[
d[\text{IP}_4]/dt = k_{bc}[\text{IP}_3] - k_{ca}[\text{IP}_4]. \tag{2.75}
\]

The total amount of available inositol phosphates was assumed to have a constant value (3.3 \text{ \textmu M}) during a normal pacemaker activity. The basal level of [\text{IP}_3] (less than 50 nM) calculated from our simulation was in a range similar to the value (10 \pm 3 nM) obtained by Wang et al. (1995).

\[
[\text{PI}_{\text{total}}] = [\text{PIP}_2] + [\text{IP}_3] + [\text{IP}_4]. \tag{2.76}
\]
The rate constants of IP$_3$ production were set to be voltage- and Ca$^{2+}$-dependent (Vergara et al. 1985; Best & Bolton 1986; Biden & Wollheim 1986; Takazawa et al. 1990; Wang et al. 1995; Ganitkevich & Isenberg 1996; Goto et al. 2004). The voltage dependence was given to the forward and backward rate constants to reproduce the voltage-dependent activation of the autonomous inward currents obtained by Goto et al. (2004). The forward rate constant was set to increase exponentially as the membrane potential goes into the positive direction, while the backward rate constant was set to decrease exponentially. The Ca$^{2+}$ dependence was also given to the forward rate constant of IP$_3$ production. Since the Hill function has been used to describe the Ca$^{2+}$ dependence on the IP$_3$ production (Biden & Wollheim 1986; Takazawa et al. 1990; Wang et al. 1995; Allen et al. 1997), the half-activation by Ca$^{2+}$ ($K_{m_{Cal}}$) was taken to model the IP$_3$ production. $K_{m_{Cal}}$ was set to 10 nM, which is close to the basal level of [IP$_3$] obtained by Wang et al. (1995). As for the remaining part of IP$_3$ metabolic pathways, we set the rate constants empirically to fit the shape of experimentally obtained pacemaker potentials.

$$\begin{align*}
    k_{ab} &= 0.2 \exp((V + 48.5)/18.1) \frac{[\text{Ca}^{2+}]_i}{[\text{Ca}^{2+}]_i + K_{m_{Cal}}}, \\
    k_{ba} &= 0.5 \exp((V + 100)/(-28.5)), \\
    k_{bc} &= 0.004, \\
    k_{ca} &= 0.0035 \exp((V + 100)/(-25.5)).
\end{align*}$$

3. Results

(a) Reconstruction of pacemaker activity in the mouse ICCs

We simulated the electrical activity of ICCs from mouse small intestine with the integration of various cellular events as described in §2. As a result, spontaneous and regularly firing APs (pacemaker potential) were successfully reproduced (figure 1). The resting and overshoot potentials were around $-78$ and $+1.2$ mV, respectively. The duration of depolarizations was around 639 ms as measured at 50% repolarization. The frequency of pacemaker potentials was around 20 min$^{-1}$. Compared with the experimental recordings by Goto et al. (2004), the frequency is higher in our model cell (20 versus 16.2 min$^{-1}$, the model simulation versus the experimental recording, respectively) and the duration (639 versus 489.1 ms) is also longer in the model cell. The repetitive firing of APs was very stable throughout the simulation experiment, which exceeded the equivalent of 10 min on a cellular time-scale.

A single pacemaker potential from the mouse ICCs was compared with that from the model simulation (figure 2). The shape of the pacemaker potential is apparently similar between the two recordings (figure 2, left panel).

Both the recordings show a low resting potential, abrupt depolarization and repolarization, and a long plateau characteristic of ICCs (Dickens et al. 2000). The rising phase of the spontaneous depolarization was also compared between

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the two recordings in an expanded view (figure 2, right panel). The maximum rate of rise is 7.1 V s⁻¹ in the experimental recording, while it is 4.32 V s⁻¹ in the model simulation. Goto et al. (2004) reported other cell groups with a different range of the maximum rate of rise (3.6 ± 0.7 V s⁻¹ at around −22.2 mV, n = 5) in the same study. The duration at 50% repolarization was also different for the recording in figure 2 (489.1 ± 32.8 versus 269.5 ± 29.7 ms, n = 5).

(b) Activation of the $I_{AI}$ by depolarization

The different value of the maximum rate of rise among the cells indicates that the amplitude of the pacemaker inward current is also variable. Figure 3a shows the activation of inward currents in a mouse ICC by depolarization with a conventional voltage-clamp protocol. The holding potential was −80 mV. As a 30 mV depolarization with a duration of 200 ms was applied, a large inward current was evoked after a delay of approximately 20 ms. This large inward current was also evoked with smaller amplitude by larger depolarizations. Interestingly, this inward current showed an autonomous time course lasting...
about 500 ms, irrespective of the duration of the depolarizing pulse. The delay in the development of inward current was shortened with a larger depolarization. The time course of the inward current after repolarization to the holding potential was also not changed. Based on these characteristic features, Goto et al. (2004) called this inward current the autonomous inward current ($I_{AI}$). Figure 3b shows the recordings made by the model simulation. The model simulation also shows similar inward currents in response to a series of membrane depolarizations. The time course of current relaxation was found to differ between the experimental and simulation recordings. The current size also differed between the two, with smaller amplitude in the simulation recording. The latter observation indicates that the difference in the values for the maximum rate of rise (see figure 2) between two recordings is derived from the different size of the $I_{AI}$.

(c) Changes in cellular parameters during the occurrence of a pacemaker potential

Figure 4 shows the time-dependent changes in cellular parameters such as membrane currents, $[Ca^{2+}]_i$, $[IP_3]$, $[Ca^{2+}]_{rel}$ and availability of the IP$_3R$ channel ($P_{o,IP_3R}$). The data demonstrate that the major conductance causing...
a pacemaker depolarization in ICCs is $I_{A1}$ and that the conductance causing the repolarization is $I_{K1}$ (figure 4a). The close relationship among the time courses of membrane potential, $[IP_3]$, $[Ca^{2+}]_i$ and $P_{o,IP3R}$ favours the hypothesis that cyclic changes in IP$_3$ and Ca$^{2+}$ might play a central role in the pacemaker mechanism (Van Helden et al. 2000; Goto et al. 2004).

4. Discussion and conclusions

Our model faithfully reproduces the pacemaker activity of the intestinal ICCs. Spontaneous and repetitive firing of APs continues as long as the simulation is running. The initial triggering event is thought to begin with the Ca$^{2+}$ leak from the SR and the DHP-resistant pathway ($I_{VDDR}$) (Kim et al. 2002). In fact, the removal of the Ca$^{2+}$ leak from the SR and plasma membrane abolished the spontaneous activity of the model (data not shown). A local rise in Ca$^{2+}$ concentration near the SR activates the IP$_3$-mediated Ca$^{2+}$ release (Berridge 1990; Bezprozvanny et al. 1991; Jaffe 1991; Iino et al. 1993; Marchant & Taylor 1997; Taylor & Laude 2002). In addition, cytosolic Ca$^{2+}$ concentration enhances the IP$_3$ production by the activation of IP$_3$ 3-kinase (Biden & Wollheim 1986; Takazawa et al. 1990; Wang et al. 1995). Taken together, Ca$^{2+}$ release and IP$_3$ production work in a cooperative manner and make a positive feedback to
increase \([\text{Ca}^{2+}]_i\). An increase in the \(\text{Ca}^{2+}\) concentration in the subsarcolemmal region to a threshold value activates \(\text{Ca}^{2+}\)-activated channels, which depolarize the cell membrane (Hirst et al. 2002; Goto et al. 2004). The membrane depolarization has an additional effect of enhancing \(\text{IP}_3\) production (Vergara et al. 1985; Best & Bolton 1986; Wang et al. 1995; Ganitkevich & Isenberg 1996; Goto et al. 2004). A prolonged increase of \(\text{IP}_3\) production by membrane depolarization creates the plateau phase of the pacemaker potential. The depletion of the \(\text{Ca}^{2+}\) pool and the gradual decrease of the available \(\text{IP}_3\) pool are thought to terminate the plateau phase. A repolarization to the threshold level finally activates the inward rectifier \(\text{K}^+\) channel, which restores the membrane potential to the resting level.

Although our model reproduces the pacemaker activity faithfully, it is unclear whether the parameter values used here appropriately reflect the real dynamics and homeostasis of a cell. There are so many interactions between parameter values that it is nearly impossible to obtain a single solution set that fits the experimental results. Each component of modelling should be validated by carefully designed experiments. For example, the effect of \([\text{Ca}^{2+}]_{\text{rel}}\) on the recovery of \(\text{IP}_3\) receptor channel from the inactivated state is based on many cardiac models in which there is more direct evidence for SR \(\text{Ca}^{2+}\)-load dependence on ryanodine release channels (Matsuda & Noma 1984). It should be validated experimentally. Even though our model needs additional validation and improvement in the near future, it is thought that the model has a value in that it provides a system to test the accumulated results and hypotheses on the pacemaker activity of ICCs.

Editors’ note

Please see also related communications in this focussed issue by Steinberg et al. (2006) and Terashima et al. (2006).

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