Ionic Mechanisms of Cardiac Cell Swelling Induced by Blocking Na+/K+ Pump As Revealed by Experiments and Simulation

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Although the Na+/K+ pump is one of the key mechanisms responsible for maintaining cell volume, we have observed experimentally that cell volume remained almost constant during 90 min exposure of guinea pig ventricular myocytes to ouabain. Simulation of this finding using a comprehensive cardiac cell model (Kyoto model incorporating Cl− and water fluxes) predicted roles for the plasma membrane Ca2+-ATPase (PMCA) and Na+/Ca2+ exchanger, in addition to low membrane permeabilities for Na+ and Cl−, in maintaining cell volume. PMCA might help maintain the [Ca2+] gradient across the membrane though compromised, and thereby promote reverse Na+/Ca2+ exchange stimulated by the increased [Na+], as well as the membrane depolarization. Na+ extrusion via Na+/Ca2+ exchange delayed cell swelling during Na+/K+ pump block. Supporting these model predictions, we observed ventricular cell swelling after blocking Na+/Ca2+ exchange with KB-R7943 or SEA0400 in the presence of ouabain. When Cl− conductance via the cystic fibrosis transmembrane conductance regulator (CFTR) was activated with isotretinoin during the ouabain treatment, cells showed an initial shrinkage to 94.2 ± 0.5%, followed by a marked swelling 32.0 ± 4.9% after 90 min drug application. Concomitantly with the onset of swelling, a rapid jump of membrane potential was observed. These experimental observations could be reproduced well by the model simulations. Namely, the Cl− efflux via CFTR accompanied by a concomitant cation efflux caused the initial volume decrease. Then, the gradual membrane depolarization induced by the Na+/K+ pump block activated the window current of the L-type Ca2+ current, which increased [Ca2+]i. Finally, the activation of Ca2+-dependent cation conductance induced the jump of membrane potential, and the rapid accumulation of intracellular Na+ accompanied by the Cl− influx via CFTR, resulting in the cell swelling. The pivotal role of L-type Ca2+ channels predicted in the simulation was demonstrated in experiments, where blocking Ca2+ channels resulted in a much delayed cell swelling.

INTRODUCTION

The Na+/K+ pump is one of the constitutive proteins present in almost all mammalian cells. It maintains concentration gradients of Na+ and K+ across the cell membrane by exchanging three Na+ for two external K+ ions, using energy from the hydrolysis of one ATP molecule. Thereby, it has an essential role in regulating the cell volume (Balshaw et al., 2001). Recently, Armstrong (2003) proposed a simple mathematical model of cell volume regulation in skeletal muscle, which satisfies the predictions of Donnan equilibrium (Boyle and Conway, 1941). We have also constructed a basic model of Cl− homeostasis and cell volume regulation in cardiac ventricular cells (Terashima et al., 2006), which was composed of background Na+, K+, and Cl− membrane conductances, as well as the Na+/K+ pump and NKCC1 (Na+/K+2 Cl− cotransporter 1). According to these model analyses, the mechanisms of cell volume regulation are detailed as follows. The [K+] gradient, created by the Na+/K+ pump across the membrane, is the main determinant of a negative Vm. This negative Vm expels Cl− out of the cell through Cl− channels, compensating for the continuous Cl− influx via Cl−-coupled transporters, such as NKCC1. Thereby, the pump maintains cellular osmolarity at the physiological level to keep the cell volume intact. Accordingly, the time course of cell swelling caused by blocking the Na+/K+ pump largely...
depends on redistribution of Cl− across the membrane and therefore on the membrane Cl− permeability, since the overall total ion flux must obey macroscopic electroneutrality. Membrane Na+ permeability also determines the time course of cell swelling indirectly, through impeding the redistribution of K+ across the membrane during the Na+/K+ pump block (Terashima et al., 2006). This general mechanism was experimentally supported by Dierkes et al. (2006) in leech Retzius neurons. Contrary to the above theoretical expectations, however, it is well known that cardiac cell volume hardly changes during Na+/K+ pump blockade (Pine et al., 1980; Drewnowska and Baumgarten, 1991; Wright and Rees, 1998). However, mechanisms underlying this preservation of cell volume have not yet been elucidated on a quantitative basis.

To clarify mechanisms of cellular responses, such as cell volume regulation, which are accomplished by the complex interactions of many factors, mathematical model analysis is indispensable. To date, several computer models of membrane excitation have been published for ventricular myocytes (see Noble and Rudy, 2001 for review; and the Kyoto model proposed by Matsuoka et al., 2003, 2004). However, neither Cl− homeostasis nor cell volume regulation was included in these models. Previously, we extended the algorithm of calculating the Cl− and water fluxes established in the basic model to the comprehensive cardiac cell model, which included mechanisms underlying membrane excitation, intracellular Ca2+ dynamics, and contraction (Terashima et al., 2006). In the present study, we have fine tuned this model, based on the additional experimental data of cardiac ion and water fluxes, and analyzed the mechanisms of cell volume regulation by conducting computer simulations in parallel with experimental validation, by measuring cell area, as an index of cell volume, and Vm using a voltage-sensitive fluorescent dye, di-8-ANEPPS. We confirmed that the Na+/K+ pump block caused a strong membrane depolarization, accompanied by minimal change in cell volume, as described in the literatures (Pine et al., 1980; Drewnowska and Baumgarten, 1991; Wright and Rees, 1998). In addition, we found that cell volume changed in a biphasic manner when the membrane Cl− conductance was increased by pharmacological intervention. The involvement of multiple factors such as PMCA, Na+/Ca2+ exchange, L-type Ca2+ channels, and Ca2+-activated background cation current I(Ca), in cell volume regulation were quantitatively examined, in addition to the contributions of membrane Na+, K+, and Cl− conductances.

MATERIALS AND METHODS

Parameter Set in the Kyoto Model

Based on additional experimental data, we have improved our ventricular cell model (Terashima et al., 2006), which had been implemented with algorithms of Cl− and water fluxes. A complete list of equations composing the Kyoto model is available in Tables S1–S13 (available at http://www.jgp.org/cgi/content/full/jgp.200609646/DC1), together with abbreviations. Fig. 1 shows a schematic diagram of the Kyoto model. The channels and transporters focused in the present study are boxed off. The extracellular ion concentrations were set (in mM) for [Na+]o at 141, [K+]o 5.4, [Ca2+]o 1.8, [Cl−]o 140, and [La]o 10, except for the simulations of drug effects (Fig. 3 B and Figs. 4, 5, and 8), where [Ca2+]o was set at 20 μM. The steady state is well established with 2.5 Hz stimulation, as well as under quiescent conditions. The ion concentrations under these conditions shown in Table I are physiologically relevant, as described in detail below. Additionally, the configurations of the action potential, intracellular Ca2+ transient and major ionic currents under 2.5-Hz stimulation shown in Fig. 2, confirm the validity of membrane excitability, as formulated in the Kyoto model. All calculations were conducted using the Euler method, with the adaptive time step with simBio (Sarai et al., 2006b), and the source code of the Kyoto model is available from http://www.sim-bio.org/. All parameters related to volume regulation, except the background Cl− conductance and NKCC1, are based on experimental findings as described below.

(1) Water Flux. The water flux Jw is driven by the difference in total ion concentrations ([Total ions], sum of Na+, K+, Cl−, Ca2+, and LA across the cell membrane; see Table S4 in the online supplemental material).

\[ J_{\text{w}} = 0.0029 \times ([\text{Total ions}]_{\text{o}} - [\text{Total ions}]_{\text{i}}) \]

A hydraulic conductivity of 0.0029 μm2/mM/ms is determined from experimental recordings of osmotic cell swelling (Wang et al., 1997; Sasaki et al., 1999), which is comparable to the experimental data reported by Suleymanian and Baumgarten (1996) and by Ogura et al. (2002).

(2) Cl− Fluxes. The total Cl− flux is composed of ICFTR, IIRCC, ICP, and NKCC1. The kinetic schemes of these components are the same as used by Terashima et al. (2006), but their amplitudes have been slightly modified by model fitting to new experimental findings in the present study. There are large variations in the ICFTR current density in accordance with the variable expression levels of CFTR mRNA in guinea pig ventricular myocytes (James et al., 1996). Here, we set PCFTR so that the current density of ICFTR is within the range of the reported values (James et al., 1996) (see Table S8 in the online supplemental material). The Kyoto model can reproduce the current–voltage relationships recorded at various concentrations of isoproterenol in guinea pig ventricular cells obtained by Tareen et al. (1992) (see Fig. 5 in Terashima et al., 2006).

The volume-regulated Cl− current IIRCC is a major factor determining the regulatory volume decrease, albeit that the extent of spontaneous regulatory volume decrease is quite small in guinea pig ventricular myocytes (Yamamoto et al., 2004). We have formulated IIRCC according to the epithelial model of Strieter et al. (1990) and the experimental data reported by Shuba et al. (1996) in guinea pig ventricular myocytes (see Fig. 2 in Terashima et al., 2006 and see Table S8 in the online supplemental material). PIRCC is set so that it can reproduce well the volume–osmolarity relationship described in guinea pig ventricular myocytes by Sasaki et al. (1999) (see Fig. S1 in the online supplemental material).

There are few data estimating the amplitude of the background membrane Cl− conductance, ICL, and flux via Cl−–coupled transporters in guinea pig ventricular myocytes. We tentatively used the NKCC1 kinetic model developed by Benjamin and Johnson.
to represent Cl−-coupled transporters (Terashima et al., 2006). The amplitude factors $I_{Ca}$ and $M_{NaCCX}$ were model adjusted, so that the almost constant cell volume observed during Na+/K+ pump inhibition in this study could be reproduced (Figs. 3 and 4) (see Tables S8 and S10 in the online supplemental material).

As a result, [Cl−] in the Kyoto model is 30 mM (Table I), comparable to 20–30 mM [Cl−], in guinea pig ventricular myocytes (Nakao and Gadsby, 1989) can also be reproduced by the Kyoto model (Fig. S2 in the online supplemental material). In addition, higher stimulus frequencies increase [Cl−], in the Kyoto model (Table I), consistent with the rapid pacing-induced accumulation of Cl− in dog atrial myocytes (Akai et al., 2003).

(3) Background Na+ and K+ Fluxes. We set the amplitude factors of the background cation conductance $I_{bg}$ and Na+/K+ pump based on the experimental reports of Kiyosue et al. (1993) and Nakao and Gadsby, (1989) (Tables S9 and S10 in the online supplemental material). An $I_{bg}$ of −0.18 pA/pF at −50 mV in the Kyoto model is the same as that reported in guinea pig ventricular myocytes (Kiyosue et al., 1993). In addition, the current density and current−voltage relationship of the Na+/K+ pump in guinea pig ventricular myocytes (Nakao and Gadsby, 1989) can also be reproduced by the Kyoto model (Fig. S2 in the online supplemental material; the current density at 0 mV is 1.3 pA/pF in guinea pig ventricular myocytes and 1.2 pA/pF in the Kyoto model). The current densities of the Na+/K+ pump and background inward Na+ current determined by Gao et al. (1995) in the guinea pig ventricular myocytes were 0.25 ± 0.09 pA/pF and 0.75 ± 0.26 pA/pF at −60 mV, respectively. The corresponding values of the Kyoto model are 0.44 pA/pF and 0.42 pA/pF, respectively, both of which are in the same range as the experimental values. [Na+], at rest and with 2.5 Hz stimulus in the Kyoto model are 2.13 and 6.37 mM, respectively (Table I), both of which are within the range of the experimental data of ~5 and 6.5 mM obtained in guinea pig papillary muscle (Wang et al., 1988).

The equations for $I_{Ca}$ are the same as that used in Matsuoka et al. (2003), where the dependence on [Ca2+]i, determined in guinea pig ventricular myocytes (Ehara et al., 1988) was used (see Table S9 in the supplemental material). The maximum activation of $I_{Ca}$ produces 8.6 nS/cell, which is within the lower range of the experimental estimation from single channel recordings, 7.2 to 72 nS/cell (Ehara et al., 1988).

(4) Ca2+ Fluxes. Influx through L-type Ca2+ channels is balanced by the efflux via Na+/Ca2+ exchange and PMCA in cardiac myocytes. Recently, model analysis by Sarai et al. (2006a) suggested that involvement of PMCA was required to simulate the rhythmic contractions observed in Ncx1-knockout mouse. We used the same kinetic model for PMCA as reported by Luo and Rudy (1994) (see Table S10 in the online supplemental material). Most recently, the relative contribution of PMCA to the total Ca2+ extrusion was reported to be 7.1% in guinea pig ventricular myocytes at 37°C (Mackiewicz and Lewartowski, 2006). Based on this report, we tentatively assumed that the contribution of PMCA to total Ca2+ extrusion is 9% (10% of Na+/Ca2+ exchanger).

It is well established that the activity of the Na+/Ca2+ exchanger is regulated by both [Na+]i and [Ca2+]i, in the excised membrane patch (Hilgemann et al., 1992a,b), and that these Nα-dependent inactivation and Ca2+-dependent activation mechanisms are relevant to [Ca2+]i, dynamics in guinea pig ventricular myocytes (Fujioka et al., 2000; Kuratomi et al., 2003). The kinetic model of these regulatory processes proposed by Fujioka et al. (2000) is used in the present study (see Table S10 in the online supplemental material), but the stoichiometry is tentatively fixed to the standard 3 Na+:1 Ca2+ exchange. Using this model, the $I_{NaCa}$ current density of 4.4 pA/pF at 50 mV is comparable to that observed in guinea pig ventricular myocytes, 4–6 pA/pF assuming the experimental condition of high [Ca2+]i (0.8 μM) (Lin et al., 2006). Furthermore, the partial inactivation of Na+/Ca2+ exchange at resting [Ca2+]i prevents [Ca2+]i from decreasing too low during quiescence. In the Kyoto model, [Ca2+]i are 21 and 46 nM at rest and at end-diastole during 2.5 Hz stimulation, respectively (Table I), both of which are only slightly smaller than those measured in

<table>
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<th>TABLE I</th>
<th>Steady-state Variables</th>
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<td>Steady-state values</td>
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<tr>
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<td>without stimulation</td>
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<tr>
<td>$V_m$ (mV)</td>
<td>−87.80</td>
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<tr>
<td>[Na+]i (mM)</td>
<td>2.13</td>
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<tr>
<td>[K+]i (mM)</td>
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<tr>
<td>[Cl−]i (mM)</td>
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<tr>
<td>[Ca2+]i (mM)</td>
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<tr>
<td>[LA]i (mM)</td>
<td>118.83</td>
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<tr>
<td>[ATP]i (mM)</td>
<td>6.97</td>
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<td>$V_o$ (μM)</td>
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activation of ICFT.

In the present model simulation, spontaneous Ca\(^{2+}\) release is attenuated tentatively by removing the \([Ca^{2+}]_o\)-dependent term of \(k_i\), the rate constant determining the transition from the closed state to the open state in the RyR channel model (see Table S11 in the online supplemental material). As described in Results, the spontaneous and repetitive cell contractions were largely inhibited during the application of ouabain by omitting CaCl\(_2\) from the external saline, while some cells still contracted repetitively at the terminal phase of the simultaneous application of ouabain and isoproterenol. In accordance with the changes in RyR channel model, several amplitude factors regarding the Ca\(^{2+}\) handling have been slightly modified, so as not to affect the configurations of both the action potential and intracellular Ca\(^{2+}\) transient.

**Simulation of Drug Effects on Ventricular Myocytes**

According to the experimental conditions used in the present study, all simulations of drug effects (Fig. 3 B and Figs. 4, 5, and 8) were performed with no stimulus and a \([Ca^{2+}]_o\) of 20 \(\mu\)M, assuming this level of residual Ca\(^{2+}\) in the nominally Ca\(^{2+}\)-free Tyrode solution.

The dose-dependent inhibition of the Na\(^+/K^+\) pump by ouabain is determined based on the report by Baker et al. (1969) (Table S10 in the online supplemental material), a blockade as much as 98.5\% of the Na\(^+/K^+\) pump by 40 \(\mu\)M ouabain is comparable to the experimental data, where the complete inhibition of the Na\(^+/K^+\) pump was accomplished by >50 \(\mu\)M ouabain in the guinea pig ventricular myocytes (Gao et al., 2002).

In the present study, we used 1 \(\mu\)M isoproterenol in experiments and simulations. Isoproterenol of 1 \(\mu\)M induces nearly full activation of \(I_{K_{EPI}}\) in the Kyoto model. Isoproterenol has also been reported to activate \(I_{Cal}\) by three- to fourfold (Delpech et al., 1995; Findlay, 2002). For the activation of the L-type Ca\(^{2+}\) channel, we simply assume a threefold increase in the amplitude of \(I_{Cal}\) during \(\beta\)-adrenergic stimulation.

It should be noted that changes in [ATP], in the Kyoto model were negligibly small, in all conditions of simulation performed in the present study, excluding possible modulations of ATP-dependent mechanisms, such as the active ion transporters, the L-type Ca\(^{2+}\) channel, and the CFTR Cl\(^-\) channel.

**Single Cell Preparation**

Single ventricular myocytes were obtained by treating guinea pig hearts with collagenase as previously described (Powell et al., 1980; Wang et al., 1997; Sasaki et al., 1999). The experimental protocols were approved by the Animal Research Committee in the Graduate School of Medicine, Kyoto University.

**Solutions and Drugs**

To avoid spontaneous contractions caused by blocking Na\(^+/K^+\) pump, a nominally Ca\(^{2+}\)-free Tyrode solution was used. The Ca\(^{2+}\)-free Tyrode solution contained (in mM) NaCl 140, NaH\(_2\)PO\(_4\) 0.33, KCl 5.4, MgCl\(_2\) 0.45, glucose 5.5, and HEPES 5 (pH 7.4). The modified D-MEM solution was prepared by adding 20 mM NaCl and 25 mM HEPES to D-MEM (without NaHCO\(_3\); MP Biomedicals) (pH 7.4).

Drugs used were 40 \(\mu\)M ouabain (Na\(^+/K^+\) pump inhibitor; Sigma-Aldrich), 1 \(\mu\)M isoproterenol (\(\beta\)-adrenergic agonist for CFTR activation; Sigma-Aldrich), 20 \(\mu\)M KB-R7943 (Na\(^+/Ca^{2+}\) exchange blocker; Tocris Cookson Inc.), 1 \(\mu\)M SEA0400 (Na\(^+/\) Ca\(^{2+}\) exchange blocker; a gift from Taisho Pharmaceutical Company, Ltd.), and 5 \(\mu\)M nifedipine (L-type Ca\(^{2+}\) channel blocker; Sigma-Aldrich). KB-R7943, SEA0400, and nifedipine were dissolved in DMSO as stock solutions and diluted with Tyrode solution. The final concentration of DMSO was 0.1%.

**Measurement of Cell Area as an Index of Cell Volume**

The measurement of cell area provides an estimate for determining changes in volume of cardiac myocytes (Yamamoto et al., 2001; Walsh and Zhang, 2005). The myocytes were settled onto the glass bottom of a recording chamber mounted on an inverted microscope (Eclipse TE2000; Nikon) equipped with a 40× oil objective and were superfused with nominally Ca\(^{2+}\)-free Tyrode solution at 36–37°C. Images of the cell were obtained every 30 s by a cooled CCD digital camera (ORCA-ER; Hamamatsu Photonics), and the cell area was calculated using Image-Pro Plus version 5.1 software (Media Cybernetics). The cell images during cell contractions were excluded from the data.
Measurement of $V_m$ Using a Voltage-sensitive Dye, di-8-ANEPPS

Changes in $V_m$ were monitored by dual-wavelength ratio imaging of a voltage-sensitive fluorescent dye, di-8-ANEPPS (Invitrogen) (Bedlack, 1992). Although small downward drifts in the fluorescence ratio of di-8-ANEPPS were observed during the long-term measurements (see Fig. 3 A), this method has advantages over the standard method of measuring $V_m$ using glass electrodes. First, we can completely avoid the ion diffusion through the electrode tip between the pipette solution and the cytosol. Also, continuous recording of $V_m$ is feasible by using the $V_m$-sensitive dye. Myocytes were loaded with di-8-ANEPPS according to the manufacturer’s protocol. In brief, myocytes were incubated for 20 min in the modified D-MEM solution containing 1 μM di-8-ANEPPS at 16°C and rinsed twice. Fluorescent image pairs of single cells with excitation at 440 ± 10 and 535 ± 25 nm from a 100-W xenon arc lamp were acquired (575 nm dichroic mirror and emission of >590 nm) every 30 s using the cooled CCD digital camera. For each cell, a pair of background images, which were adjacent to the target cell, but contained no other cells, was taken for background subtraction. The fluorescent ratio $R$ is defined as the ratio of the fluorescence intensity at an excitation wavelength of 440 nm to that at 535 nm. The use of the ratiometric method avoids any effects of small variations in dye concentration on the fluorescence results. To convert the fluorescence ratio $R$ to a value in mV, whole-cell voltage clamp was performed using a pipette solution containing (in mM) 145 KCl, 1 MgCl$_2$, 1 EGTA, and 5 HEPES (pH 7.2). In brief, the $V_m$ was clamped to different levels ranging from −120 to +20 mV, and pairs of fluorescence images were taken at each potential and analyzed as described above. The calibration curve is shown in Fig. S3. The R values plotted against the $V_m$ showed a linear relationship (correlation coefficient, $r = 0.84$, $P < 0.01$), while a relatively large variation in the absolute value of $R$ for each $V_m$ was observed between the individual myocyte (see large error bars in the Fig. S3). The $V_m$ value obtained in each experiment was calculated using the following equation:

$$V_m = \frac{R - 0.80}{8.95 \times 10^{-3}}.$$  

Statistics

Results are represented as mean ± SEM. Statistical analyses were performed by one-factor ANOVA using StatView (SAS Institute Inc.). Multiple and two-group comparisons were performed according to Student-Newman-Keul’s method and Student’s $t$ test, respectively. $P < 0.05$ was considered significant.

Online Supplemental Material

Lists of abbreviations and all equations used in the Kyoto model are available as Tables S1–S13 in the online supplemental material (http://www.jgp.org/cgi/content/full/jgp.200609646/DC1). In addition, the steady-state variables such as ionic currents, flux, and metabolite concentrations are in Table S14. The volume-osmolarity relationship (Fig. S1) and the current–voltage relationship of Na$^+$/K$^+$ pump (Fig. S2) in the Kyoto model, and the calibration curve for di-8-ANEPPS (Fig. S3) are also presented.

RESULTS

Maintenance of Cell Volume during the Membrane Depolarization Induced by Applying Ouabain to Ventricular Myocytes

Membrane depolarization takes the pivotal role in coupling blockade of the Na$^+$/K$^+$ pump to cell swelling (Armstrong 2003; Terashima et al., 2006). In experiments shown in the top panel of Fig. 3 A, $V_m$ was measured with or without the continuous application of 40 μM ouabain. The control without ouabain was shown as mean ± SEM. Treatment with 40 μM ouabain caused an obvious membrane depolarization, with a large variation in the time course among different cells, as shown by five representative recordings in the top panel of Fig. 3 A. On average, the peak depolarization to $-40.0 \pm 7.1$ mV was attained 66.5 ± 6.8 min ($n = 10$) after applying ouabain. This average value is in agreement with the reported $V_m$ ($\sim 33$ mV at 1 h or $\sim 20$ mV at 2 h after

Figure 3. Experimental recordings of the cell area and $V_m$ during the Na$^+$/K$^+$ pump block (A) and simulation of the Na$^+$/K$^+$ pump block using the Kyoto model (B). (A) Top panel shows measurements of $V_m$ in five representative cells (colored lines) with 40 μM ouabain. The control records obtained in the absence of ouabain were averaged and represented as mean ± SEM ($n = 4$). Measurements of cell area ($n = 5$) with 40 μM ouabain were shown in the middle panel with different colors. The control records obtained in the absence of ouabain were presented as mean ± SEM in the bottom panel ($n = 5$). The joining error bars represent contour of the plots. When the error bars are not shown, they are smaller than the symbols. (B) The simulation of the corresponding experimental condition was performed by applying 40 μM ouabain at time 0 to the Kyoto model and the time courses of $V_m$ expressed in percent of original $V_m$ (top) and $V_a$ (bottom) were shown.
applying ouabain) measured by penetrating the intra-cellular microelectrode into the full-thickness section of ventricular wall, where the variation in \( V_m \) or membrane conductance, if any, among intact cells should be automatically averaged through the electrical coupling via gap junction (Pine et al., 1980). In contrast to \( V_m \), no obvious cell swelling was observed as shown in the middle panel of Fig. 3 A, where five representative recordings of the cell area during Na\(^+\)/K\(^+\) pump block are shown. In all experiments, a continuous but small decrease was observed. This decrease was also observed in the control experiments without applying ouabain (Fig. 3 A, bottom). The cell area at 90 min was 98.6 ± 0.6% of the original area in the control group (\( n = 5 \)) and 96.9 ± 0.7% in the ouabain-treated group (\( n = 5 \)). These findings clearly indicate that cell swelling should be quite small during the 90-min application of ouabain, which is in agreement with previous reports (Pine et al., 1980; Drewnowska and Baumgarten, 1991). The median time course of depolarization was simulated well by the Kyoto model, as shown in Fig. 3 B. Indeed, the increase in cell volume is much delayed and small, compared with the membrane depolarization. Cell volume is 101.3% of the original value 90 min after applying 40 \( \mu \)M ouabain, indicating quite a small change in cell area.

Role of Membrane Cl\(^-\) and Na\(^+\) Conductances in Cell Volume Regulation As Revealed by Simulation

Mechanisms underlying the almost constant cell volume accompanying the variable time courses of membrane depolarization during the Na\(^+\)/K\(^+\) pump blockade were quantitatively examined by varying the membrane conductances for Cl\(^-\) and Na\(^+\) in the simulation. In Fig. 4 A, membrane Cl\(^-\) conductance was changed by scaling \( P_{Cl} \), and \( MNKCC1 \) simultaneously to keep control [Cl\(^-\)], at the physiological level of 30 mM. In Fig. 4 B, membrane Na\(^+\) conductance was changed by varying the magnitude of \( P_{Na} \). It is obvious that the cell swells to a larger extent with increased membrane Cl\(^-\) and Na\(^+\) conductances during Na\(^+\)/K\(^+\) pump block, and that the rate of cell swelling is mainly determined by the membrane Cl\(^-\) conductance (Fig. 4 A, top). Decreasing the Na\(^+\) conductance delays the onset of cell swelling, leaving the rate of swelling nearly constant (Fig. 4 B, top). These results support the notion that the rate of Cl\(^-\) influx is rate limiting in cell swelling, since the overall total flux obeys macroscopic electroneutrality (Armstrong 2003; Terashima et al., 2006). Although there are limited experimental data on the magnitude of membrane background Cl\(^-\) conductance, the simulation of experimental results (Fig. 3 A) using the comprehensive cell model suggests that the membrane Cl\(^-\) conductance is small in guinea pig ventricular myocytes.

It is also obvious that the experimental variation in the time course of membrane depolarization can be explained by varying the membrane Na\(^+\) conductance, not by varying the Cl\(^-\) conductance, as shown in the bottom panels of Fig. 4. Here, membrane depolarization is caused by the redistribution of K\(^+\) across the membrane, i.e., by changes in the K\(^+\) equilibrium potential. To satisfy electroneutrality, this redistribution of K\(^+\) should be coupled with the counter movement of Na\(^+\) or with the parallel flux of Cl\(^-\). The electrochemical driving force for Cl\(^-\) is much smaller than that for Na\(^+\), because \( E_{Cl} \) deviates only slightly from \( V_m \) by the Cl\(^-\) influx through NKCC1. Therefore, changing the membrane Cl\(^-\) conductance causes only marginal variations in the rate of K\(^+\) redistribution, while that of Na\(^+\) greatly affects the K\(^+\) redistribution. The accelerated membrane depolarization...
caused by increasing $P_{\text{NSC}}$ shortens the critical time when $V_m$ becomes more positive than $E_{CP}$. This results in the overall Cl$^-$ influx through channels as well as NKCC1, and the simultaneous cation influx, since the total ionic flux obeys macroscopic electroneutrality. Thereby, intracellular osmolarity increases to evoke cell swelling (Fig. 4 B, top). These simulations suggest that the experimental variation in $V_m$ (Fig. 3 A) might be caused by variation in membrane Na$^+$ conductance between 0.5 and 2 times the control. Indeed, Kiyosue et al. (1993) reported a considerable variation in the current density of the background Na$^+$ conductance among myocytes dissociated by enzymatic treatment. The largest cell swelling in this range of $P_{\text{NSC}}$ is 2.3% at 90 min, which is within the extent of the spontaneous decrease in cell area observed in experiments (Fig. 3 A). It may be possible that relatively small volume changes could not be resolved by measuring the cell area.

**Role of PMCA and Na$^+$/Ca$^{2+}$ Exchanger in Cell Volume Regulation As Predicted by Simulation**

The variation in $V_m$ at the quasi-steady state established by the continuous application of ouabain, as seen in the bottom panel of Fig. 4 B, is caused by a trace of Na$^+$/K$^+$ pump activity (1.5% of control) remaining during drug application. [Na$^+$], at 120 min are 142.3, 140.8, 137.2, 127.0, and 110.3 mM at ×5, 2, 1, 0.5, and 0.2 of $P_{\text{NSC}}$ in the simulation, respectively. Indeed, the variation is much smaller when the Na$^+$/K$^+$ pump activity is completely blocked (unpublished data). This prompted us to examine the role of the other active transporter PMCA in determining $V_m$ as well as cell volume during Na$^+$/K$^+$ pump block. To visualize the role of PMCA, simulations were performed with and without PMCA. As clearly demonstrated in Fig. 5 B, [Ca$^{2+}$], is maintained at a low level (0.18 μM) by active Ca$^{2+}$ extrusion via PMCA. Omitting PMCA from the Kyoto model results in a marked Ca$^{2+}$ accumulation (19.50 μM, Fig. 5 B) when an $I_{\text{CaL}}$, window current is induced by the membrane depolarization after ~40 min of ouabain treatment. Ca$^{2+}$ accumulation increases membrane Na$^+$ conductance, through activation of $I_{\text{NaCa}}$ (13-fold increase), resulting in the jump of $V_m$ at ~40 min, as shown in the bottom panel of Fig. 5 A. Thus, in the absence of PMCA, Na$^+$, K$^+$, and Ca$^{2+}$ are passively redistributed across the membrane, and $V_m$ as well as $E_{\text{NaCa}}$ become almost zero, resulting in a greater cell swelling (the blue line in Fig. 5 C). In contrast, PMCA maintains $E_{\text{NaCa}}$ (~−115 mV in the top panel of Fig. 5 A) more negative than $V_m$ in combination with the increased [Na$^+$], and membrane depolarization even after 40 min. Thereby, Na$^+$ is extruded via reversed Na$^+$/Ca$^{2+}$ exchange throughout the period of ouabain treatment. This reverse mode of Na$^+$/Ca$^{2+}$ exchange, in addition to less activation of $I_{\text{NaCa}}$, retards Na$^+$ accumulation within the cell and results in less membrane depolarization during Na$^+$/K$^+$ pump block (Fig. 5 A). The increase in cell volume decreased to about half by PMCA, after 120 min inhibition of the Na$^+$/K$^+$ pump (Fig. 5 C). We conclude that PMCA partially substitutes for the Na$^+$/K$^+$ pump in extruding Na$^+$ out of the cells by using the ATP. Our experimental finding that no contracture was observed during Na$^+$/K$^+$ pump inhibition supports this hypothesis.

The above working hypothesis was tested, experimentally, as shown in Fig. 6. When 20 μM KB-R7943, a blocker of Na$^+$/Ca$^{2+}$ exchange (Elias et al., 2001; Iwamoto, 2004), was applied 60 min after ouabain treatment, the cell started to swell (Fig. 6 A, top). Essentially the same result was obtained by using a more specific and

![Figure 5](image-url)
potent blocker of Na+/Ca2+ exchange, SEA0400 (Matsuda et al., 2001; Iwamoto, 2004) (Fig. 6 A, bottom). To evaluate the effects of these Na+/Ca2+ exchange blockers superimposed on the continuous decrease in cell area, the cell area at the end of 60 min treatment with blockers was normalized with respect to the cell area at the beginning of treatment with these drugs (Fig. 6 B, shaded bars). For comparison, the cell area at 120 min normalized by the 60-min area without the drug treatment (open bar) or only with ouabain (filled bar) are shown. As shown in Fig. 6 B, blocking Na+/Ca2+ exchange significantly increased the cell area, indicating operation of the reversed mode of exchanger during ouabain treatment. These experimental results verify the theoretical predictions of the model simulation.

Experimental Demonstration of Cell Swelling Induced by Blocking Na+/K+ Pump in the Presence of an Increased Cl− Conductance

So far it has been demonstrated that the cell swelling induced by Na+/K+ pump block is largely dependent on the magnitude of the membrane Cl− conductance and that this Cl− conductance is extremely small in the guinea pig ventricular myocytes. If so, activation of CFTR Cl− channels by β-adrenergic stimulation should induce cell swelling (Tatsumi et al., 2002). To confirm this assumption, we examined the effects of simultaneous application of 40 μM ouabain and 1 μM isoproterenol on guinea pig ventricular myocytes. As shown in Fig. 7, cell swelling occurred after a delay of 52.0 ± 4.9 min (n = 8). The change of cell area was biphasic. First it gradually decreased to 94.2 ± 0.5% of the original cell area and then increased finally to 104.9 ± 3.1% 30 min after starting to swell (Fig. 7 A). In addition, a sudden jump of \( V_m \) after the gradual depolarization to the peak potential of −30.8 ± 4.4 mV (n = 5) was observed (Fig. 7 B). It should be noted that there were no significant differences between the time when the rapid swelling started, 52.0 ± 4.9 min, and the time when the sudden jump of \( V_m \) occurred, 58.8 ± 2.2 min (P = 0.80). These findings were contrasting to the data in Fig. 3 A, where no change in cell area and no \( V_m \) jump were observed.

Simulation of the Biphasic Volume Change Induced by Ouabain and Isoproterenol and its Experimental Validation

The Kyoto model could reproduce the experimentally observed volume change, a gradual decrease followed by a rapid increase with a sudden jump in \( V_m \) (Fig. 8, A and D). The initial decrease in cell volume is due to enhanced Cl− efflux through CFTR, activated (from 0 to 1.3 nS) by applying 1 μM isoproterenol (Fig. 8 B, see red line). It should be noted that Cl− efflux is accompanied by an equal amount of cation efflux (expressed as a sum of fluxes of Na+, K+, and 2 · Ca2+) satisfying macroscopic electroneutrality (Fig. 8 C). The Cl− efflux, however, is truncated by the continuous membrane depolarization caused by the loss of K+ during the Na+/K+ pump block, and thereby the initial volume decrease is saturated (Fig. 8, A, B, and D). The minimum cell volume is 94.4% of the original volume (Fig. 8 A). During the course of membrane depolarization, the window current of L-Type Ca2+ channel, which is also magnified threefold by β-adrenergic stimulation, is gradually activated over the potential range less negative than −40 mV, as indicated in Fig. 8 E, resulting in a rapid accumulation of [Ca2+]i at 42 min (Fig. 8 F). This increase of [Ca2+]i, activates \( I_{CaL} \) (Fig. 8 G) and triggers a sudden jump in \( V_m \) to a level more positive than \( E_{Cl} \) (Fig. 8 D). Thereby, the rapid and marked cell swelling is induced through an accumulation of Cl−, as well as Na+ and K+ (Fig. 8, A and B).

To experimentally test the working hypothesis of the involvement of \( I_{CaL} \) in initiating the rapid swelling, we applied a specific blocker of L-type Ca2+ channels, nifedipine, simultaneously with ouabain and isoproterenol, to the myocytes (Fig. 9). It was obvious that the
swelling phase was significantly depressed in the presence of 5 μM nifedipine (cell area at 30 min after the start of swelling was 98.0 ± 0.7% and 104.9 ± 3.1% of original area with and without nifedipine, respectively, n = 8–9, P = 0.037), leaving the first gradual shrinkage phase intact (average cell area of 95.5 ± 0.7% original; Fig. 9). These results strongly supported the notion that [Ca²⁺]i accumulation via the window current of L-type Ca²⁺ channel caused the rapid swelling triggered by membrane depolarization, as predicted by the model simulation.

**DISCUSSION**

The Extremely Small Membrane Cl⁻ Permeability Contributes To Stabilizing the Volume of Guinea Pig Ventricular Myocytes

The present study conducted experiments as well as simulations using our comprehensive cardiac cell model, the Kyoto model, to analyze mechanisms underlying cell volume regulation in guinea pig ventricular myocytes. The general mechanisms of cell volume regulation (Armstrong 2003; Terashima et al., 2006) were applicable to the cardiac myocytes, in which cell swelling was minimal during Na⁺/K⁺ pump block for 1–2 h. It is the extremely small membrane Cl⁻ conductance that is responsible for the negligible cell swelling of guinea pig ventricular myocytes. The rate of [Cl⁻]i accumulation calculated in the simulation (0.025 mM/min) is ~100-fold smaller than the experimental rate (2.7 ± 1.6 mM/min) described in the leech Retzius neurons, where significant swelling was observed during ouabain treatment (Dierkes et al., 2006). The notion that membrane Cl⁻ conductance in the guinea pig ventricular myocytes should be small is consistent with the experimental findings that no obvious effect of varying the extra- or intracellular Cl⁻ concentrations was observed on the membrane current–voltage relationship in the

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**Figure 7.** Experimental recordings of the cell area and Vm during the Na⁺/K⁺ pump block with increased membrane Cl⁻ conductance. The ventricular cells were incubated with 40 μM ouabain as well as 1 μM isoproterenol, and the cell area (A) and Vm (B) were measured in different cells (n = 5–8). Mean ± SEM of time and cell area measured just before a start of obvious cell swelling were superimposed on the representative recording (A). Mean ± SEM of time just after the Vm jump and that of peak Vm were superimposed on the representative recording (B).

**Figure 8.** Model prediction for the experimental observations in Fig. 7. At time 0, 40 μM ouabain and 1 μM isoproterenol were applied simultaneously. Changes in Vm (A), amounts of intracellular ions expressed as Eq \times 10^{−12} (B), amounts of intracellular cation (blue) or anion (red) (C), Vm (D), ICaL (E), [Ca²⁺] (F), and Il(Ca) (G) were demonstrated. Since the present simulation ignores minor changes in anion concentrations accompanying the Ca²⁺ binding to proteins and Ca²⁺ flux into SR, there is a slight difference between total amounts of cation and anion.
Figure 9. Experimental validation of the $I_{\text{CaL}}$ involvement in the cell swelling as predicted in Fig. 8. 5 μM nifedipine was applied to block $I_{\text{CaL}}$ simultaneously with 40 μM ouabain and 1 μM isoproterenol. A representative record is shown.

to our knowledge, this is the first evidence that high-$\text{Na}^+$ resistance was established in the present study by using the Na pump exchanger. This function of PMCA in cell volume regulation is the reverse mode of the Na pump but can be explained by assuming incomplete block of the Na/K pump in a variety of experimental ischemia. In fact, our simulations as well as our experiments (Fig. 3) indicated that $[\text{Na}^+]_o$ could be very variable with different Na conductances, provided that only a few percent of Na/K pump activity remains during pump inhibition. The variation in the magnitude of membrane background Na conductance in isolated myocytes (Fig. 3) may also affect the time course of Na overload during ischemia. However, we do not know whether the variation in the background Na conductance (Matsuda, 1983; Ehara et al., 1988; Kiyosue et al. 1993) occurs in the intact heart or the variation was artifactually caused during enzymatic cell dissociation.

The Positive Feedback Cycle between $I_{\text{CaL}}$ and $I_{\text{KCa}}$ in Determining Cell Swelling

The $\text{Ca}^{2+}$-activated background conductances have been frequently discussed in relation to arrhythmic membrane excitation (Carmeliet, 1999), such as the delayed membrane depolarization, but rarely analyzed in cell volume regulation. The present experimental and simulation study using the comprehensive cardiac cell model has disclosed the cascade of events leading to the final cell swelling during Na/K pump block: gradual membrane depolarization due to redistribution of K across the membrane, opening of the window $I_{\text{CaL}}$, an increase in $[\text{Ca}^{2+}]_i$, activation of $I_{\text{KCa}}$, accelerated cell volume constant (Fujise et al., 1991; Milanick and Frame, 1991; Blaustein and Lederer, 1999). It may be concluded that cell volume regulation through PMCA is one of the fundamental mechanisms for all kinds of mammalian cells, although it is not prominent under normal physiological conditions. Considering the high $[\text{Ca}^{2+}]_i$, caused by the Na/K pump block as well as the low $[\text{Ca}^{2+}]_o$ of 20 μM in our experimental conditions, compared with the values in resting myocytes with a normal $[\text{Ca}^{2+}]_o$ of 1.8 mM, it may be possible that the transport via PMCA was enhanced under the present experimental conditions.

The involvement of PMCA in volume regulation may also be variable in magnitude between different experimental conditions, depending on stimulation by $\text{Ca}^{2+}$-calmodulin and PKA (Dixon and Haynes, 1989). In the simulation shown in Fig. 5, the magnitude of $\text{Ca}^{2+}$ efflux via PMCA during ouabain treatment increased to as much as 5 μmol/liter cytosol/s. This value is comparable to the experimental estimation of the maximum rate of $\text{Ca}^{2+}$ extrusion via PMCA with calmodulin, 2.43 μmol/liter cytosol/s (Dixon and Haynes, 1989; Bers, 2003), suggesting that the present estimation of the PMCA contribution is relevant.

It is well known that a large variation exists in the extent of Na overload during ischemia in the isolated perfused heart (Hartmann and Decking, 1999; Varadarajan et al., 2001; Bak and Ingwall, 2003). This variation may be explained by assuming incomplete block of the Na/K pump in a variety of experimental ischemia. In fact, our simulations as well as our experiments (Fig. 3) indicated that $[\text{Na}^+]_o$ could be very variable with different Na conductances, provided that only a few percent of Na/K pump activity remains during pump inhibition. The variation in the magnitude of membrane background Na conductance in isolated myocytes (Fig. 3) may also affect the time course of Na overload during ischemia. However, we do not know whether the variation in the background Na conductance (Matsuda, 1983; Ehara et al., 1988; Kiyosue et al. 1993) occurs in the intact heart or the variation was artifactually caused during enzymatic cell dissociation.

absence of β-adrenergic stimulation (guinea pig ventricular myocytes; Bahinski et al., 1989; Matsuoka et al., 1990). We conclude that cardiac cells have a great advantage in protecting themselves against swelling under certain pathological conditions where the activity of the Na/K pump is impaired.

PMCA in Conjunction with $\text{Na}^+$/Ca$^{2+}$ Exchanger Plays an Important Role in Cell Volume Regulation

It has been assumed that PMCA plays only a complementary role for the Na/Ca exchanger in extruding Ca$^{2+}$ out of cardiac cells (Bers et al., 1996; Choi and Eisner, 1999; Mackiewicz and Lewartowski, 2006). However, we recently proposed by means of a quantitative model analysis, that the Ca$^{2+}$ dynamics could be retained within normal bounds by increasing PMCA activity even in the absence of the Na/Ca$^{2+}$ exchanger, as achieved experimentally in the NCX knockout mouse (Sarai et al., 2006a). The present study Na/K pump blockade also reveals a pivotal role for PMCA in impeding membrane depolarization, as well as cell swelling by preventing a massive Na$^+$ accumulation in conjunction with the reverse mode of the Na/Ca$^{2+}$ exchanger. This function of PMCA in cell volume regulation was established in the present study by using the Na/Ca$^{2+}$ exchange blockers KB-R7943 and SEA0400. To our knowledge, this is the first evidence that highlights the coordinated action of PMCA and the Na/Ca$^{2+}$ exchanger in the regulation of cardiac cell volume. Interestingly, an essentially similar mechanism has been described in a particular kind of red blood cell that has no detectable Na/K pump but can maintain cell volume. In these cells, Ca$^{2+}$ is extruded primarily by a PMCA with a much higher activity than in other cells, such as cardiac cells. Then driven by the energy stored in the electrochemical Ca$^{2+}$ gradient, the Na/Ca$^{2+}$ exchanger serves the unusual role of extruding Na$^+$ in exchange for entering Ca$^{2+}$, to keep the energy stored in the electrochemical Ca$^{2+}$ gradient, as achieved experimentally in the NCX knockout cell that has no detectable Na/pump. A representative record is shown.
depolarization, reversion from Cl− efflux to influx through Cl− channels, increase of intracellular osmolarity due to the Cl− and accompanied cation influxes, and final net influx of water. Once swelling commences, volume-dependent activation of $I_{VRRC}$ further accelerates the swelling. Note that the activation of $I_{CaL}$ and $I_{Ca(T)}$ occurs via a positive feedback cycle mediated by the increase in [Ca2+]i, and membrane depolarization, causing the rapid jump in membrane depolarization (Fig. 7). The application of isoproterenol in the present study simply accelerated the process and enhanced the cell swelling by magnifying the Ca2+ influx via $I_{CaL}$ as well as the membrane Cl− conductance. Thus, harmful effects of β-adrenergic stimulation are evident during metabolic impairment.

Activation of an additional conductance by increased [Ca2+]i, has been described in cardiac myocytes, the Ca2+-activated Cl− channel $I_{Ca(T)}$. Although this channel has been extensively characterized using dog and rabbit ventricular myocytes (Zygmunt and Gibbons, 1991; Collier et al., 1996), there is a little information about $I_{Cl(T)}$ in the guinea pig ventricular myocyte, and it is still controversial with regard to the magnitude of guinea pig $I_{Cl(T)}$ (Sipido et al., 1995; Nakajima et al., 2002). To examine the possible involvement of $I_{Cl(T)}$ in volume regulation, we tentatively implemented $I_{Cl(T)}$ into the Kyoto model and performed simulations of blocking the Na+/K+ pump. The extent of cell swelling was simply augmented with increasing magnitude of $I_{Cl(T)}$ during ouabain application. The rapid inactivation gate, as suggested by the very brief outward current at the beginning of a depolarizing pulse (Sipido et al., 1993), though not included in the above model simulation, should be beneficial in avoiding the unfavorable role of $I_{Cl(T)}$ in volume regulation.

Limitations in the Model Simulations and Experiments

The feedback cycle of formulating a working hypothesis through simulation and validating the hypothesis by conducting new experiments, as exemplified in this study, does facilitate understanding of complex physiological and pathophysiological functions, involving the interactions of numerous molecular mechanisms. In this respect, the mechanisms of cell swelling suggested in the present study should be further tested by detailed experimental investigations.

Although our basic model specified for volume regulation disclosed the principal mechanisms (Terashima et al., 2006), a deeper insight into the complicated interactions among multiple molecular mechanisms requires a comprehensive cell model. The Kyoto model has already included most of the ion channels and the major ion transporters on the cardiac cell membrane, mechanisms for the Ca2+ dynamics performed by SR, the intracellular Ca2+ buffers, such as calmodulin and troponin, and the model of oxidative phosphorylation in mitochondria (Korzeniowsky and Zoladz, 2001). The parameters have been adjusted to simulate different kinds of experimental findings, such as membrane excitability, frequency-dependent variation of intracellular ion concentrations, excitation-contraction coupling, and volume regulation, as demonstrated in previous publications (Matsuoka et al., 2003, 2004; Terashima et al., 2006). Thereby, we could derive a conclusion that Ca2+ dynamics had quite an important role in cardiac cell volume regulation indirectly, through affecting Na+ homeostasis. However, obvious limitations in the model analysis of cell swelling are imposed by the lack of calculating the intermediate metabolite concentrations, as well as pH homeostasis, which is composed of acid and base transporters (cotransport or antiport with Na+ or Cl−) and buffering reactions. As shown in Fig. 3 B, a more or less cell swelling (1.3% increase at 90 min) is inevitable in the Kyoto model when the Na+/K+ pump is blocked, whereas no obvious volume increase was detected experimentally (Fig. 3 A). This discrepancy also proposes working hypotheses that additional mechanisms, such as changes in pH homeostasis, metabolism, or unknown osmolyte transporters, which are beyond the scope of the present study, might be involved in keeping the cell volume constant.

We failed to observe a contracture at the onset of rapid swelling during the application of ouabain and isoproterenol (Fig. 7). This finding is inconsistent with the prediction of a [Ca2+]i accumulation temporarily as large as 4 μM (Fig. 8), which should have caused a contracture. We might have missed the contracture because of the 30-s duration between capturing cell images. Alternatively, the [Ca2+]i-dependent activation of PMCA via calmodulin kinase II or activation via PKA, which were not implemented in the Kyoto model, might have prevented [Ca2+]i, to rise and cause a contracture.

It should finally be commented that limitations in interpreting the experimental findings also arise by the method of measuring the cell volume, as well as $V_w$. The measurement of cell area as an index of the cell volume might underestimate the real cell volume change, because it is likely that the cell thickness continuously changed with the experimental period (Sasaki et al., 1999). The apparent hyperpolarization detected by the dye (Fig. 7) on the application of isoproterenol is also different from the membrane depolarization measured by the patch clamp method (Sasaki et al., 1999). However, it is obvious in the top panel of Fig. 3 A that the measured $V_w$ tended to decrease continuously, even without any drugs (~15 mV decreases during the 90-min measurement). If this trend was subtracted as a measurement error, the sudden jump of $V_w$ shown in Fig. 7 B should have started at ~40 mV, which was near the threshold $V_w$ for $I_{CaL}$ window current in the Kyoto model.
We thank Professor T. Powell and Professor D. Hilgemann for their critical reading of the manuscript and fruitful suggestions, and Professor C.H. Leem for determining the membrane hydraulic conductivity.

This study was supported by the Leading Project for Biosimulation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Olaf S. Andersen served as editor.

Submitted: 8 August 2006
Accepted: 28 September 2006

REFERENCES


Table S1. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>$CF_X$</td>
<td>dependency of ion flux on $V_m$ (constant field equation)</td>
</tr>
<tr>
<td>$C_m$</td>
<td>membrane capacitance (pF)</td>
</tr>
<tr>
<td>$E_K$</td>
<td>equilibrium potential for $K^+$ (mV)</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday’s constant, 96.4867 C/mmol</td>
</tr>
<tr>
<td>$f_{Ca}$</td>
<td>fraction of Na$^+$/Ca$^{2+}$ exchanger that regulatory Ca$^{2+}$ bound</td>
</tr>
<tr>
<td>$f_{PKA}$</td>
<td>fraction of cAMP-activated PKA</td>
</tr>
<tr>
<td>$G_x$</td>
<td>conductance (pA/mV)</td>
</tr>
<tr>
<td>$I_{X}$</td>
<td>ion X component of current $I_a$ (pA/pF)</td>
</tr>
<tr>
<td>$I_{NSC}$</td>
<td>background non-selective cation current (pA/pF)</td>
</tr>
<tr>
<td>$I_{Ca}$</td>
<td>background Ca$^{2+}$ current (pA/pF)</td>
</tr>
<tr>
<td>$G_{Ca}$</td>
<td>L-type Ca$^{2+}$ current (pA/pF)</td>
</tr>
<tr>
<td>$i_{Cal}$</td>
<td>single channel current of $I_{Cal}$ (pA/pF)</td>
</tr>
<tr>
<td>$I_{ATP}$</td>
<td>T-type Ca$^{2+}$ current (pA/pF)</td>
</tr>
<tr>
<td>$I_{CFTR}$</td>
<td>CFTR Cl$^-$ channel current (pA/pF)</td>
</tr>
<tr>
<td>$I_{Cl}$</td>
<td>background Cl$^-$ current (pA/pF)</td>
</tr>
<tr>
<td>$I_{Cl(Ca)}$</td>
<td>Ca$^{2+}$-activated Cl$^-$ channel current (pA/pF)</td>
</tr>
<tr>
<td>$I_{ext}$</td>
<td>current applied through the electrode (pA/pF)</td>
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<td>$I_{KI}$</td>
<td>inward rectifier K$^+$ current (pA/pF)</td>
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<tr>
<td>$I_{KATP}$</td>
<td>ATP-sensitive K$^+$ current (pA/pF)</td>
</tr>
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<td>$I_{Kp}$</td>
<td>voltage-dependent K$^+$ current (plateau current) (pA/pF)</td>
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<td>$I_{Kr}$</td>
<td>delayed rectifier K$^+$ current, rapid component (pA/pF)</td>
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<td>$I_{Ks}$</td>
<td>delayed rectifier K$^+$ current, slow component (pA/pF)</td>
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<td>$I_I$</td>
<td>total of background current (time-independent) (pA/pF)</td>
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<td>Ca$^{2+}$-activated background cation current (pA/pF)</td>
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<td>Na$^+$ current (pA/pF)</td>
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<td>Na$^+$/Ca$^{2+}$ exchange current (pA/pF)</td>
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<td>Na$^+$/K$^+$ pump current (pA/pF)</td>
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<td>$I_{netX}$</td>
<td>sum of current components carried by ion X (pA/pF)</td>
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<td>$I_{PMCA}$</td>
<td>PMCA current (pA/pF)</td>
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<tr>
<td>$I_{SR}$</td>
<td>Ca$^{2+}$ leak from SR (pA/pF)</td>
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<tr>
<td>$I_{SRRyR}$</td>
<td>Ca$^{2+}$ release through RyR channel in SR (pA/pF)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$I_{SR}T$</td>
<td>Ca$^{2+}$ transfer from SR uptake site to release site (pA/pF)</td>
</tr>
<tr>
<td>$I_{SR}U$</td>
<td>Ca$^{2+}$ uptake into SR (pA/pF)</td>
</tr>
<tr>
<td>$I_o$</td>
<td>transient outward current (pA/pF)</td>
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<tr>
<td>$I_{tot}$</td>
<td>total current of ion channels and ion exchangers (pA/pF)</td>
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<td>$I_{VRCC}$</td>
<td>VRCC current (pA/pF)</td>
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<tr>
<td>$J_{NKCC1}$</td>
<td>ion flux via NKCC1 (x amol/msec), x = 1 for Na$^+$ and K$^+$, x = 2 for Cl$^-$ : amol = 10$^{-18}$ mol</td>
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<td>$J_{water}$</td>
<td>water flux across the cell membrane (m$^3$/msec)</td>
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<tr>
<td>$k, \alpha, \beta, \lambda$</td>
<td>rate constants (/msec)</td>
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<tr>
<td>$K_X, K_{oX}$</td>
<td>association constant for ion X (/mM), dissociation constant for ion X (mM)</td>
</tr>
<tr>
<td>LA</td>
<td>impermeable large anion (mM)</td>
</tr>
<tr>
<td>$M_{NKCC1}$</td>
<td>an amplitude factor for NKCC1 (amol)</td>
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<tr>
<td>NKCC1</td>
<td>Na$^+$/K$^+$/2Cl$^-$ cotransporter 1</td>
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<tr>
<td>PI</td>
<td>inorganic phosphate (mM)</td>
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<tr>
<td>PKA</td>
<td>cAMP-dependent protein kinase A (mM)</td>
</tr>
<tr>
<td>PMCA</td>
<td>plasma membrane Ca$^{2+}$-ATPase</td>
</tr>
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<td>$P_x$</td>
<td>convert factor (pA/pF/mM) for CF$^x$</td>
</tr>
<tr>
<td>$p(X)$</td>
<td>probability of state X in a scheme of multiple state transitions</td>
</tr>
<tr>
<td>$R$</td>
<td>Gas constant, 8.3143 C mV/K/mmol</td>
</tr>
<tr>
<td>RyR</td>
<td>ryanodine receptor</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>$T$</td>
<td>absolute temperature, 310 K</td>
</tr>
<tr>
<td>$V_i$</td>
<td>cell volume accessible for ion diffusion, 100 $\cdot$ 20 $\cdot$ 8 $\cdot$ 0.8 = 12800 m$^3$</td>
</tr>
<tr>
<td>$V_m$</td>
<td>membrane potential (mV)</td>
</tr>
<tr>
<td>$V_{mit}$</td>
<td>volume of mitochondria (m$^3$)</td>
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<tr>
<td>VRCC</td>
<td>volume-regulated Cl$^-$ channel</td>
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<td>$V_{rel}$</td>
<td>volume of SR release site (m$^3$)</td>
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<tr>
<td>$V_t$</td>
<td>total cell volume, 100 $\cdot$ 20 $\cdot$ 8 = 16000 m$^3$</td>
</tr>
<tr>
<td>$V_{up}$</td>
<td>volume of SR uptake site (m$^3$)</td>
</tr>
<tr>
<td>$X_i$</td>
<td>intracellular amount of substance X (amol)</td>
</tr>
<tr>
<td>$X_o$</td>
<td>extracellular amount of substance X (amol)</td>
</tr>
<tr>
<td>$[X]_i$</td>
<td>intracellular concentration of substance X (mM)</td>
</tr>
<tr>
<td>$[X]_o$</td>
<td>extracellular concentration of substance X (mM)</td>
</tr>
<tr>
<td>$y_n$</td>
<td>probability for two states transition (0 $\leq$ $y_n$ $\leq$ 1)</td>
</tr>
<tr>
<td>$z_X$</td>
<td>valence of ion X</td>
</tr>
</tbody>
</table>

**Contraction**
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ExternalForce</td>
<td>external force ($= \text{ForceCB} + \text{ForceEcomp}, \text{mN/mm}^2$)</td>
</tr>
<tr>
<td>ForceEcomp</td>
<td>elastic component of force (mN/mm$^2$)</td>
</tr>
<tr>
<td>ForceCB</td>
<td>cross-bridge force (mN/mm$^2$)</td>
</tr>
<tr>
<td>h</td>
<td>cross-bridge elongation (m)</td>
</tr>
<tr>
<td>hSML</td>
<td>half sarcomere length ($hSML = X + h, \text{m}$)</td>
</tr>
<tr>
<td>T</td>
<td>fraction of contraction unit with free troponin C</td>
</tr>
<tr>
<td>TCa</td>
<td>fraction of contraction unit with troponin C bound to Ca$^{2+}$</td>
</tr>
<tr>
<td>TCa*</td>
<td>fraction of contraction unit with troponin C bound to Ca$^{2+}$ and attached cross bridge (force generator)</td>
</tr>
<tr>
<td>T*</td>
<td>fraction of contraction unit with attached cross bridge and with free troponin C (force generator)</td>
</tr>
<tr>
<td>X</td>
<td>length composed of half of the thick filament and the free portion of the thin filament, (m)</td>
</tr>
</tbody>
</table>

**Mitochondria and ATP metabolism**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[a^{2+}]$</td>
<td>reduced cytochrome $a_i$ ($a^{2+}$) (mM)</td>
</tr>
<tr>
<td>$[a^{3+}]$</td>
<td>cytochrome $a_i$ ($a^{3+}$) (mM)</td>
</tr>
<tr>
<td>$A_{\text{ox}}$</td>
<td>ratio of oxidized $a^{3+}$ to $a^{2+}$</td>
</tr>
<tr>
<td>$[A_{\text{total}}]_i$</td>
<td>total concentration of intracellular adenine (mM)</td>
</tr>
<tr>
<td>$[C_{\text{total}}]$</td>
<td>total concentration of creatine (mM)</td>
</tr>
<tr>
<td>$[P_{\text{total}}]$</td>
<td>total concentration of phosphate (mM)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP-AK}}]$</td>
<td>ATP production rate by adenylate kinase (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP-CaPump}}]$</td>
<td>rate of ATP consumption by SR Ca$^{2+}$ pump (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP-CK}}]$</td>
<td>ATP production rate by creatine kinase (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP-contraction}}]$</td>
<td>rate of ATP consumption by contraction (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP-NAK}}]$</td>
<td>rate of ATP consumption by Na$^+$ pump (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP}}]_i$</td>
<td>rate of cytoplasmic ATP change (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ATP-total}}]_i$</td>
<td>rate of mitochondria total ATP production (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{ADP}}]_i$</td>
<td>rate of cytoplasmic ADP change (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{c^{2+}}}]_i$</td>
<td>rate of reduced cytochrome $c^{2+}$ ($c^{2+}$) production (mM/msec)</td>
</tr>
<tr>
<td>$d[H_{\text{limit}}]$</td>
<td>rate of mitochondria H$^+$ change (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{NADH}}]_i$</td>
<td>rate of NADH production (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{phosphocreatine}}]_i$</td>
<td>rate of cytoplasmic phosphocreatine change (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{PI}}]_i$</td>
<td>rate of mitochondria total PI production (mM/msec)</td>
</tr>
<tr>
<td>$d[A_{\text{UQH}_2}]_i$</td>
<td>rate of reduced ubiquinone (UQH$_2$) production (mM/msec)</td>
</tr>
<tr>
<td>$G_{C1}$</td>
<td>thermodynamic span of complex I (mV)</td>
</tr>
<tr>
<td>$G_{C3}$</td>
<td>thermodynamic span of complex III (mV)</td>
</tr>
<tr>
<td>$G_{SN}$</td>
<td>thermodynamic span of ATP synthase (mV)</td>
</tr>
<tr>
<td>$E_{ma}$</td>
<td>cytochrome $a_i$ redox potential (mV)</td>
</tr>
</tbody>
</table>
cytochrome c redox potential (mV)
NAD redox potential (mV)
ubiquinone redox potential (mV)
buffering capacity for H⁺ in mitochondria
ratio of mitochondria volume to cell volume, 0.23
rate of substrate dehydrogenation in mitochondria (mM/msec)
rate of Complex I (mM/msec)
rate of Complex III (mM/msec)
rate of Complex IV (mM/msec)
rate of ATP synthase (mM/msec)
rate of ATP/ADP exchanger (mM/msec)
rate of phosphate carrier (mM/msec)
rate of proton leak (mM/msec)

Table S2. Cell volume

<table>
<thead>
<tr>
<th></th>
<th>( V_i ) (m³)</th>
<th>( V_{rel} ) (m³)</th>
<th>( V_{app} ) (m³)</th>
<th>( C_m ) (pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular cell</td>
<td>(100 · 20 · 8) · 0.8</td>
<td>0.02 · ( V_i )</td>
<td>0.05 · ( V_i )</td>
<td>211.2</td>
</tr>
</tbody>
</table>

Table S3. Ca²⁺-binding proteins

<table>
<thead>
<tr>
<th></th>
<th>( K_d ) (( k_f/k_b )) (mM)</th>
<th>( k_f ) (1/mM/msec)</th>
<th>( k_b ) (1/msec)</th>
<th>Total concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TroponinC</td>
<td>7.70 · 10⁻⁴</td>
<td>39</td>
<td>0.03</td>
<td>70 · 10⁻³</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>2.38 · 10⁻³</td>
<td>100</td>
<td>0.238</td>
<td>50 · 10⁻³</td>
</tr>
<tr>
<td>Calsequestrin</td>
<td>8.00 · 10⁻¹</td>
<td></td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Table S4. Calculation of the membrane potential and internal ion concentrations

Membrane potential

\[
dV_m / dt = -(I_{tot} + I_{ext})
\]

\[
I_{tot} = I_{Na} + I_{Ca} + I_{Cl} + I_{K} + I_{Na} + I_{K} + I_{in} + I_{NaCa} + I_{PMCA}
\]

\[
I_{f} = I_{NSC} + I_{K} + I_{Ca} + I_{KATP} + I_{Cl} + I_{RCC} + I_{EFTR}
\]

Water flux
\[ \text{[Total ions]}_i = [\text{Na}^+]_i + [\text{K}^+]_i + [\text{Cl}^-]_i + [\text{Ca}^{2+}]_i + [\text{LA}]_i \]

\[ \text{[Total ions]}_o = [\text{Na}^+]_o + [\text{K}^+]_o + [\text{Cl}^-]_o + [\text{Ca}^{2+}]_o + [\text{LA}]_o \]

\[ J_{\text{water}} = 0.0029 \cdot (\text{[Total ions]}_i - \text{[Total ions]}_o) \]

\[ \frac{dV_i}{dt} = J_{\text{water}} \]

**Internal ion concentrations**

\[ [X]_i = \frac{X_i}{V_i} \]

\[ [X]_o = \frac{X_o}{V_o} \]

\[ \frac{d\text{Na}_i}{dt} = - C_m \cdot \frac{I_{\text{netNa}}}{z_{\text{Na}}} / F + J_{\text{NKCC1}} \]

\[ \frac{d\text{K}_i}{dt} = - C_m \cdot (I_{\text{netK}} + I_{\text{ext}}) / z_{\text{K}} / F + J_{\text{NKCC1}} \]

\[ \frac{d\text{Cl}_i}{dt} = C_m \cdot \frac{I_{\text{netCl}}}{z_{\text{Cl}}} / F + 2 \cdot J_{\text{NKCC1}} \]

\[ \frac{d\text{Ca}_i}{dt} = - C_m \cdot (I_{\text{netCa}} - I_{\text{SR RyR}} - I_{\text{SR U}} - I_{\text{SR L}}) / z_{\text{Ca}} / F + (d(p(T)) + d(p(T^*)))[\text{TroponinC}] / dt \cdot V_i \]

Free Ca\(^{2+}\) is calculated with instantaneous equilibrium for Ca\(^{2+}\) binding to calmodulin

\[ I_{\text{netNa}} = I_{\text{NaNa}} + I_{\text{CaLCa}} + I_{\text{toNa}} + I_{\text{KsNa}} + I_{\text{NSCNa}} + I_{\text{IL(Ca)Na}} \]

\[ I_{\text{netK}} = I_{\text{K1}} + I_{\text{Kr}} + I_{\text{ATP}} + I_{\text{CaK}} + I_{\text{CaK}} + I_{\text{NSK}} + I_{\text{NSK}} + I_{\text{IL(Ca)K}} + I_{\text{PMCA}} \]

\[ I_{\text{netCl}} = I_{\text{CB}} + I_{\text{VRCC}} + I_{\text{CFTR}} \]

\[ I_{\text{netCa}} = I_{\text{CaC}} + I_{\text{CaC}} + I_{\text{CaC}} - 2 \cdot I_{\text{SarCa}} + I_{\text{PMCA}} \]

**dependency of ion flux on \( V_m \) (constant field equation)**

\[ CF_x = \frac{Z_X \cdot F \cdot \Omega V_m}{R \cdot \Omega T} \left( \frac{[X]_i - [X]_o \cdot \exp\left(-Z_X \cdot F \cdot \Omega V_m\right)}{R \cdot \Omega T} \right) \]

\[
\begin{align*}
\text{Table S5. Cytoplasmic energy balance} \\
\text{creatinine kinase} \\
\text{phosphocreatine} \rightarrow ADP_{\text{total}} \xrightarrow{k_f} ATP_{\text{total}} + \text{creatine} \\
\text{creatine} \rightarrow ADP_{\text{total}} \xleftarrow{k_b} ATP_{\text{total}} + \text{phosphocreatine} \\
k_f = 16.5 \text{ (mM/msec)}, k_b = 9.67 \cdot 10^{-6} \text{ (mM/msec)}
\end{align*}
\]

\[ d[\text{ATP}_{\text{CK}}] = k_f \cdot [\text{ADP}_{\text{total}}], [\text{phosphocreatine}], - k_b \cdot [\text{ATP}_{\text{total}}], [\text{creatine}] \]

**adenylate kinase**

\[ ADP_{\text{free}} + ADP_{\text{Mg}} \xrightarrow{k_f} ATP_{\text{Mg}}, AMP_i \xleftarrow{k_b} \]

\[ k_f = 0.783 \text{ (mM/msec)}, k_b = 0.683 \text{ (mM/msec)} \]

\[ d[\text{ATP}_{\text{AK}}] = k_f \cdot [\text{ADP}_{\text{free}}], [\text{ADP}_{\text{Mg}}], - k_b \cdot [\text{ATP}_{\text{Mg}}], [\text{AMP}]_i \]
Total concentration of adenine, creatine and phosphate

\[
\begin{align*}
[A_{total}]_i &= [ATP_{total}]_i + [ADP_{total}]_i + [AMP]_i = 7.0 \\
[C_{total}]_i &= [\text{phosphocreatine}]_i + [\text{creatine}]_i = 25 \\
[P_{total}]_i &= [\text{phosphocreatine}]_i + [PI_{total}]_i + 3 \cdot [ATP_{total}]_i + 2 \cdot [ADP_{total}]_i + [AMP] + (3 \cdot [ATP_{total}]_{mit} + 2 \cdot [ADP_{total}]_{mit} + [PI_{total}]_{mit}) \cdot R_{mc} \\
&= 46.0 \\
[ATP_{free}]_i &= [ATP_{total}]_i / (1 + [Mg_{free}]_i / K_{dAT Pi}) \\
K_{dAT Pi} &= 0.024 \text{ (mM)} \\
[Mg_{free}]_i &= 4.0 \\
[ATP_{Mg}]_i &= [ATP_{total}]_i - [ATP_{free}]_i \\
[ADP_{free}]_i &= [ADP_{total}]_i / (1 + [Mg_{free}]_i / K_{dADPi}) \\
K_{dADPi} &= 0.347 \\
[ADP_{Mg}]_i &= [ADP_{total}]_i - [ADP_{free}]_i \\
\frac{d[ATP_{NaK}]}{dV} &= C_m \cdot \frac{I_{NaK}}{F \cdot 0V} \\
\frac{d[ATP_{CaPump}]}{dV} &= C_m \cdot \frac{I_{SrU}}{4nF \cdot 0V} \\
\frac{d[ATP_{Contraction}]}{dV} &= 0.4 \cdot p(TCa*) \cdot \text{TroponinC} \\
\frac{d[ATP_{total}]}{dV} &= - v_{EX} \cdot (d[ATP_{CK}] + d[ATP_{AK}] + d[ATP_{CaPump}] + d[ATP_{Contraction}]) \\
\frac{d[ADP_{total}]}{dV} &= - v_{EX} \cdot (\frac{d[ATP_{CK}]}{2} \cdot d[ATP_{CK}] + \frac{d[ATP_{NaK}]}{3} \cdot d[ATP_{NaK}] + d[ATP_{CaPump}] + d[ATP_{Contraction}]) \\
\frac{d[phosphocreatine]}{dV} &= - d[ATP_{CK}]
\end{align*}
\]

Table S6. Inward currents

\[
I_{Na} = I_{NaNa} + I_{NaK} \\
I_{NaNa} = P_{Na} \cdot CF_{Na} \cdot p(AP) \cdot y \\
I_{NaK} = 0.1 \cdot P_{Na} \cdot CF_{K} \cdot p(AP) \cdot y \\
P_{Na} = 21.67 \\
\]

Voltage-dependent gate

\[
\begin{align*}
k_{RP, AP} &= 1 / (0.1027 \cdot \exp (-V_m / 8) + 0.25 \cdot \exp (-V_m / 50)) \\
k_{AP, RP} &= 1 / (26 \cdot \exp (V_m / 17) + 0.02 \cdot \exp (V_m / 800)) \\
k_{RI, AI} &= 1 / (0.0001027 \cdot \exp (-V_m / 8) + 5 \cdot \exp (-V_m / 400)) \\
k_{AI, RI} &= 1 / (1300 \cdot \exp (V_m / 20) + 0.04 \cdot \exp (V_m / 800))
\end{align*}
\]
\begin{align*}
k_{AP, AP} &= 1 / (0.8 \cdot \exp (-V_m / 400)) \\
k_{AI, AP} &= 0.0000875 \\
k_{RP, RI} &= 0.01 / (1 + k_{AL, AP} \cdot k_{AP, RP} \cdot k_{RI, AL} / k_{AP, AI} / k_{RP, AP} / k_{AL, RI}) \\
k_{RI, RP} &= 0.01 - k_{RP, RI} \\

\text{Ultra-slow gate} \\
\begin{align*}
\alpha &= 1 / (9000000000 \cdot \exp(V_m / 5) + 8000 \cdot \exp(V_m / 100)) \\
\beta &= 1 / (0.014 \cdot \exp(-V_m / 5) + 4000 \cdot \exp(-V_m / 100)) \\

I_{CaL} &= I_{CaL, Ca} + I_{CaL, K} + I_{CaL, Na} \\
I_{CaL, Ca} &= P_{CaL, C} \cdot C_{F, Ca} \cdot p(open_{CaL}) \\
I_{CaL, K} &= 0.000365 \cdot P_{CaL, C} \cdot C_{F, K} \cdot p(open_{CaL}) \\
I_{CaL, Na} &= 0.0000185 \cdot P_{CaL, C} \cdot C_{F, Na} \cdot p(open_{CaL}) \\
P_{CaL} &= 66.0 \\
p(open_{CaL}) &= p(AP) \cdot (p(U) + p(U_{Ca})) \cdot y / (1 + (1.4 / [ATP])^3) \\

\text{Voltage-dependent gate} \\
\begin{align*}
k_{RP, AP} &= 1 / (0.27 \cdot \exp (-V_m / 5.9) + 1.5 \cdot \exp (-V_m / 65)) \\
k_{AP, RP} &= 1 / (480 \cdot \exp (V_m / 7) + 2.2 \cdot \exp (V_m / 65)) \\
k_{RI, AI} &= 1 / (0.0018 \cdot \exp (-V_m / 7.4) + 2 \cdot \exp (-V_m / 100)) \\
k_{AI, RI} &= 1 / (2200000 \cdot \exp (V_m / 7.4) + 11 \cdot \exp (V_m / 100)) \\
k_{AP, AI} &= 0.004, k_{AI, AP} = 0.001 \\
k_{RP, RI} &= 0.04 / (1 + k_{AI, AP} \cdot k_{AP, RP} \cdot k_{RI, AI} / k_{AP, AI} / k_{RP, AP} / k_{AI}) \\
k_{RI, RP} &= 0.04 - k_{RP, RI} \\

\text{Ca}^{2+}-\text{dependent gate}
\end{align*}
\end{align*}
\[ \text{Ultra-slow gate} \]

\[ \alpha_y = \frac{1}{(250000 \cdot \exp\left(\frac{V_m}{9}\right) + 58 \cdot \exp\left(-\frac{V_m}{65}\right))} \]

\[ \beta_y = \frac{1}{(8000 \cdot \exp\left(-\frac{V_m}{5.6}\right) + 0.82 \cdot \exp\left(-\frac{V_m}{250}\right))} \]

\[ \alpha_{y1} = \frac{1}{(0.019 \cdot \exp\left(-\frac{V_m}{5.6}\right) + 0.82 \cdot \exp\left(-\frac{V_m}{250}\right))} \]

\[ \beta_{y1} = \frac{1}{(40 \cdot \exp\left(V_m / 6.3\right) + 1.5 \cdot \exp\left(V_m / 10000\right))} \]

\[ \alpha_{y2} = \frac{1}{(62000 \cdot \exp\left(V_m / 10.1\right) + 30 \cdot \exp\left(V_m / 3000\right))} \]

\[ \beta_{y2} = \frac{1}{(0.0006 \cdot \exp(-V_m / 6.7) + 1.2 \cdot \exp(-V_m / 25))} \]

\[ I_{\text{Calc}} = \frac{P_{\text{Calc}} \cdot CF_{\text{Ca}} \cdot y_1 \cdot y_2}{i_{\text{Calc}}} \]

\[ P_{\text{Calc}} = 4.636 \]

\[ i_{\text{Calc}} = 0.0676 \cdot \frac{C_{\text{FCa}} \cdot [Ca^{2+}]_{cm}}{0.3 \cdot \frac{i_{\text{Calc}}}{0.143 \cdot \frac{k_{\text{CCa}} \cdot U_{\text{Ca}}}} \cdot \frac{k_{\text{CCa}} \cdot U_{\text{Ca}}}{k_{\text{CCa}} \cdot U_{\text{Ca}}}} \]

\[ \text{Table S7. Outward currents} \]

\[ I_{K1} = G_{K1} \cdot (V_m - E_K) \cdot \left( f_1^3 + 4 \cdot f_2^3 \cdot f_5 \cdot f_7 + 6 \cdot f_1^3 \cdot f_5^2 \cdot f_7^2 \right) \cdot y \]

\[ G_{K1} = 1.148 \cdot \left[ ([K^+]_o / 5.4)^{0.4} \right] \]

\[ \alpha_y = \frac{1}{(8000 \cdot \exp\left(V_m - E_K - 97\right) / 8.5) + 7 \cdot \exp\left(V_m - E_K - 97\right) / 300)} \]

\[ \beta_y = \frac{1}{(0.00014 \cdot \exp(-V_m - E_K - 97) / 9.1) + 0.2 \cdot \exp(-V_m - E_K - 97) / 500)} \]

\[ O \leftrightarrow \lambda B_1 \leftrightarrow 2 \mu B_2 \leftrightarrow 3 \lambda B_3 \leftrightarrow 4 \mu B_4 \]
\[ 
\lambda = 3 \cdot \exp(-0.048 \cdot (V_m - E_K - 10)) \cdot (1 + \exp(0.064 \cdot (V_m - E_K - 38))) 
/ (1 + \exp(0.03 \cdot (V_m - E_K - 70))) 
\]

\[ 
f_u = \lambda / (1 + \lambda) 
\]

\[ 
f_o = \lambda / (1 + \lambda) 
\]

\[ 
I_{Kr} = G_{Kr} \cdot (V_m - E_K) \cdot (0.6 \cdot y_1 + 0.4 \cdot y_2) \cdot y_3 
\]

\[ 
G_{Kr} = 0.00864 \cdot ([K^+] / 5.4)^{0.2} \]

\[ 
\alpha_{y_1} = 1 / (20 \cdot \exp(-V_m / 11.5) + 5 \cdot \exp(-V_m / 300)) 
\]

\[ 
\beta_{y_1} = 1 / (1/1000 \cdot \exp(V_m / 20)) + 1 / (2500 \cdot \exp(V_m / 20)) 
\]

\[ 
\alpha_{y_2} = 1 / (200 \cdot \exp(-V_m / 13) + 20 \cdot \exp(-V_m / 300)) 
\]

\[ 
\beta_{y_2} = 1 / (1600 \cdot \exp(V_m / 28) + 2000 \cdot \exp(V_m / 1000)) + 1 / (10000 \cdot \exp(V_m / 20)) 
\]

\[ 
\alpha_{y_3} = 1 / (10 \cdot \exp(V_m / 17) + 2.5 \cdot \exp(-V_m / 150)) 
\]

\[ 
I_{Ks} = I_{Ks,K} + I_{Ks,Na} 
\]

\[ 
I_{Ks,K} = P_{Ks,K} \cdot CF_K \cdot \phi y_1^2 \cdot \phi (0.9 \cdot y_2 + 0.1) 
\]

\[ 
I_{Ks,Na} = 0.04 \cdot P_{Ks,Na} \cdot CF_{Na} \cdot \phi y_1^3 \cdot \phi (0.9 \cdot y_2 + 0.1) 
\]

\[ 
P_{Ks,K} = 0.0382 
\]

\[ 
\alpha_{y_1} = 1 / (85 \cdot \exp(-V_m / 10.5) + 370 \cdot \exp(-V_m / 62)) 
\]

\[ 
\beta_{y_1} = 1 / (1450 \cdot \exp(V_m / 20) + 260 \cdot \exp(V_m / 100)) 
\]

\[ 
\alpha_{y_2} = 3.7 \cdot [Ca^{2+}]_i 
\]

\[ 
\beta_{y_2} = 0.004444 
\]

\[ 
I_{Na} = I_{Na,K} + I_{Na,Na} 
\]

\[ 
I_{Na,K} = P_{Na,K} \cdot CF_K \cdot \phi y_1^3 \cdot \phi y_2 
\]

\[ 
I_{Na,Na} = 0.09 \cdot P_{Na,Na} \cdot CF_{Na} \cdot \phi y_1^3 \cdot \phi y_2 
\]

\[ 
P_{Na,K} = 2.5 \times 10^{-4} 
\]

\[ 
\alpha_{y_1} = 1 / (11 \cdot \exp(-V_m / 28) + 0.2 \cdot \exp(-V_m / 400)) 
\]

\[ 
\beta_{y_1} = 1 / (4.4 \cdot \exp(V_m / 16) + 0.2 \cdot \exp(V_m / 500)) 
\]

\[ 
\alpha_{y_2} = 0.0038 \cdot \exp(-(V_m + 13.5) / 11.3) / (1 + 0.051335 \cdot \exp(-(V_m + 13.5) / 11.3)) 
\]

\[ 
\beta_{y_2} = 0.0038 \cdot \exp((V_m + 13.5) / 11.3) / (1 + 0.067083 \cdot \exp((V_m + 13.5) / 11.3)) 
\]

Table S8. Cl currents

\[ 
I_{Cl,CC} 
\]
\[ I_{VRCC} = P_{VRCC} \cdot CF_{Cl} \left( 1 + \frac{200}{1 + \exp\left( -0.001(V_i - 2170) \right)} \right) \left( \frac{1}{1 + \exp\left( -100\cdot V_{m} / 50 \right)} \right) \]

\[ P_{VRCC} = -2.0 \cdot 10^{-5} \]

\[ I_{CFTR} = P_{CFTR} \cdot CF_{Cl} \cdot p(Open) \]

\[ P_{CFTR} = -1.2 \cdot 10^{-3} \]

\[ p(Open) = \frac{\alpha_1 \cdot [ATP] \cdot \beta_2 \cdot k_1 + \alpha_2 \cdot [ATP] \cdot k_3 + \alpha_3 \cdot k_2 \cdot [ATP]^2 \cdot k_1}{\alpha_1 \cdot [ATP] \cdot \beta_2 \cdot k_1 + \alpha_1 \cdot [ATP] \cdot k_3 + \alpha_2 \cdot k_2 \cdot [ATP]^2 \cdot k_1 + \alpha_1 \cdot [ATP] \cdot \beta_2 \cdot k_2 + \alpha_1 \cdot [ATP] \cdot \beta_2 \cdot k_3 + \beta_1 \cdot \beta_2 \cdot k_1 \cdot k_2 \cdot k_3 + k_1 \cdot k_2 \cdot k_3} \]

\[ \alpha_i = 0.0756 \text{ (/mM/msec)}, \quad \alpha_2 = 1.09 \cdot 10^{-4} \text{ (/mM/msec)}, \quad \beta_i = 6.5 \cdot 10^{-3}, \quad \beta_2 = 3.0 \cdot 10^{-5} \]

\[ k_1 = 0.768 \cdot f_{PKA}, \quad k_2 = 3.85 \cdot 10^{-3}, \quad k_3 = 0.712 \cdot f_{PKA} \]

\[ f_{PKA} = \frac{1}{1 + \left( \frac{0.0029}{[cAMP]} \right)^{15}}, \quad [cAMP] = 0.000168, \quad 1 + \left( \frac{0.005}{[Isoproterenol]} \right)^{15} \]

\[ I_{ClB} = P_{ClB} \cdot CF_{Cl} \]

\[ P_{ClB} = -1.82 \cdot 10^{-4} \]

**Table S9. Background currents**

<table>
<thead>
<tr>
<th>Current</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_{NSC} )</td>
<td>( I_{NSC} = I_{NSC} \cdot Na + I_{NSC} \cdot K )</td>
</tr>
<tr>
<td>( I_{NSC} \cdot Na )</td>
<td>( I_{NSC} = P_{NSC} \cdot CF_{Na} )</td>
</tr>
<tr>
<td>( I_{NSC} \cdot K )</td>
<td>( I_{NSC} = 0.4 \cdot P_{NSC} \cdot CF_{K} )</td>
</tr>
<tr>
<td>( P_{NSC} )</td>
<td>( P_{NSC} = 5.69 \cdot 10^{4} )</td>
</tr>
<tr>
<td>( I_{Kpl} )</td>
<td>( I_{Kpl} = P_{Kpl} \cdot CF_{K} \cdot (V_{m} + 3) / (1 - \exp (- (V_{m} + 3) / 13)) )</td>
</tr>
<tr>
<td>( P_{Kpl} )</td>
<td>( P_{Kpl} = 8.333 \cdot 10^{7} \cdot (</td>
</tr>
<tr>
<td>( I_{I(Ca)} )</td>
<td>( I_{I(Ca)} = I_{I(Ca)} \cdot Na + I_{I(Ca)} \cdot K )</td>
</tr>
<tr>
<td>( I_{I(Ca)} \cdot Na )</td>
<td>( I_{I(Ca)} \cdot Na = P_{I(Ca)} \cdot CF_{Na} \cdot p(open) )</td>
</tr>
<tr>
<td>( I_{I(Ca)} \cdot K )</td>
<td>( I_{I(Ca)} \cdot K = P_{I(Ca)} \cdot CF_{K} \cdot p(open) )</td>
</tr>
<tr>
<td>( P_{I(Ca)} )</td>
<td>( P_{I(Ca)} = 7.5 \cdot 10^{-3} )</td>
</tr>
</tbody>
</table>
\[
p(\text{open}) = \frac{1}{1 + \left( \frac{0.0012}{[Ca]_i} \right)^3}
\]

\[
I_{KATP} = 0.417 \cdot [K^+]_o \cdot 0.24 \cdot (V_m - E_K) \cdot p(\text{open})
\]

\[
p(\text{open}) = \frac{0.8}{1 + \left( \frac{[ATP]}{0.1} \right)^2}
\]

\[
I_{Cab} = P_{Cab} \cdot CF_{Ca}
\]

\[
P_{Cab} = 1.5 \cdot 10^{-3}
\]

**Table S10. Transporters**

\[
\begin{array}{c}
Na^+/Ca^{2+} \text{ Exchange} \\

\begin{array}{c}
E_2Na \underset{k_5}{\xrightarrow{K_{Na}}}; E_2Ca \underset{k_6}{\xrightarrow{K_{Ca}}} \\
E_1Na \underset{k_3}{\xrightarrow{K_{Na}}}; E_1Ca \underset{k_4}{\xrightarrow{K_{Ca}}} \\
\end{array}

p(E_1\{\text{total}\})
\end{array}
\]

\[
K_{Na} = 87.5, K_{Ca} = 1.38, K_{Na} = 20.7, K_{Ca} = 0.0184
\]

\[
p(E_1Na) = 1 / (1 + (K_{Na} \cdot [Na]^3) \cdot (1 + [Ca] \cdot K_{Ca}))
\]

\[
p(E_2Na) = 1 / (1 + (K_{Ca} \cdot [Ca] \cdot (1 + [Na] \cdot K_{Na}))
\]

\[
p(E_1Ca) = 1 / (1 + (K_{Ca} \cdot [Ca] \cdot (1 + ([Na] \cdot K_{Na})^3))
\]

\[
k_i = \exp (0.32 \cdot F \cdot V_m / R / T), k_j = \exp ((0.32-1) \cdot F \cdot V_m / R / T), k_j = 1, k_j = 1
\]

\[
f_{Ca} = [Ca]^3] / ([Ca]^3] + 0.004
\]

\[
\alpha_1 = p(E_1Na) \cdot f_{Ca} \cdot 0.002 + (1 - f_{Ca}) \cdot 0.0015
\]

\[
\beta_1 = f_{Ca} \cdot 0.0012 + (1 - f_{Ca}) \cdot 5.0 \cdot 10^{-7}
\]

\[
\alpha_2 = f_{Ca} \cdot 3.0 \cdot 10^{-3} + (1 - f_{Ca}) \cdot 0.01
\]

\[
\beta_2 = f_{Ca} \cdot 0.09 + (1 - f_{Ca}) \cdot 1.0 \cdot 10^{-4}
\]

\[
d(p(E_1\{\text{total}\}) / dt = p(E_2\{\text{total}\}) \cdot (k_2 \cdot p(E_2Na) + k_4 \cdot p(E_2Ca)) + p(I_1) \cdot \beta_1 + p(I_2) \cdot \beta_2 - p(E_1\{\text{total}\}) \cdot (k_1 \cdot p(E_1Na) + k_3 \cdot p(E_1Ca) + \alpha_1 + \alpha_2)
\]

\[
d(p(I_1) / dt = p(E_1\{\text{total}\}) \cdot \alpha_1 - p(I_1) \cdot \beta_1
\]

\[
d(p(I_2) / dt = p(E_2\{\text{total}\}) \cdot \alpha_2 - p(I_2) \cdot \beta_2
\]

\[
p(E_2\{\text{total}\}) = 1 - (p(E_1\{\text{total}\}) + p(I_1) + p(I_2))
\]

\[
I_{NaCa} = 110 \cdot (k_1 \cdot p(E_1Na) \cdot p(E_2\{\text{total}\}) - k_2 \cdot p(E_2Na) \cdot p(E_2\{\text{total}\}))
\]
**Na⁺/K⁺ Pump**

\[
\begin{align*}
E_2Na & \leftrightarrow K_{\text{Na}} \rightarrow E_2 \leftrightarrow K_{\text{K}} \rightarrow E_2K \quad (1 - y) \\
& \quad k_1 \uparrow \downarrow k_2 \quad k_3 \uparrow \downarrow k_4 \quad \beta \uparrow \downarrow \alpha \\
E_1Na & \leftrightarrow K_{\text{Na}} \rightarrow E_1 \leftrightarrow K_{\text{K}} \rightarrow E_1K \quad y
\end{align*}
\]

\[K_{\text{Na}} = 69.8, \quad K_{\text{K}} = 0.258, \quad K_{\text{Na}} = 4.05, \quad K_{\text{K}} = 32.88\]

\[k_1 = 0.37 \cdot (1 / (1 + 0.094 / [\text{ATP}])), \quad k_2 = 0.04, \quad k_3 = 0.01, \quad k_4 = 0.165\]

\[p(E_1Na) = 1 / (1 + (K_{\text{Na}} / [\text{Na}^+]_i)^{1.06} \cdot (1 + ([\text{K}^+] / K_{\text{K}})^{1.12}))\]

\[p(E_2Na) = 1 / (1 + (K_{\text{Na}} / [\text{Na}^+]_i)^{1.06} \cdot (1 + ([\text{K}^+] / K_{\text{K}})^{1.12}))\]

\[p(E_1K) = 1 / ([\text{Na}^+]_i)^{1.06} \cdot (1 + ([\text{K}^+] / K_{\text{K}})^{1.12})\]

\[p(E_2K) = 1 / ([\text{Na}^+]_i)^{1.06} \cdot (1 + ([\text{K}^+] / K_{\text{K}})^{1.12})\]

\[\text{[Na}^+]_i = [\text{Na}^+] \cdot \exp (-0.82 \cdot F \cdot V_m / R / T)\]

In the reduced two-state model

\[I_{\text{Na/K}} = 11.5 \cdot (k_2 \cdot p(E_1Na) \cdot y - k_2 \cdot p(E_2Na) \cdot (1 - y)) \cdot (1 / (1 + [\text{ouabain}] / 0.0006))\]

\[\alpha = k_2 \cdot p(E_1Na) + k_4 \cdot p(E_2K)\]

\[\beta = k_1 \cdot p(E_1Na) + k_3 \cdot p(E_2K)\]

**PMCA**

\[I_{\text{PMCA}} = 0.13 \cdot (1 / (1 + 0.00005 / [\text{Ca}^{2+}]_i))\]

**Na⁺/K⁺/2Cl⁻ cotransporter**

\[
\begin{align*}
E_1 \leftrightarrow K_{\text{Na}} \rightarrow E_1NaCl & \leftrightarrow K_{\text{K}} \rightarrow E_1NaClKCl \leftrightarrow K_{\text{Cl}} \rightarrow E_1NaClKClNa \quad y \\
k_{\text{Empty}} \uparrow \downarrow k_{\text{Empty}} & \quad k_{\text{Full}} \uparrow \downarrow k_{\text{Full}} \quad \beta \uparrow \downarrow \alpha \\
E_2 & \leftrightarrow K_{\text{Cl}} \rightarrow E_2Cl \leftrightarrow K_{\text{K}} \rightarrow E_2ClKCl \leftrightarrow K_{\text{Cl}} \rightarrow E_2ClKClNa \quad (1 - y)
\end{align*}
\]

\[K_{\text{Na}} = 0.08445, \quad K_{\text{K}} = 1.16 \cdot 10^{-3}, \quad K_{\text{Cl}} = 0.05735\]

\[k_{\text{Full}} = 3.065, \quad k_{\text{Empty}} = 1.456, \quad k_{\text{Empty}} = 37.767, \quad k_{\text{Full}} = 79.522\]

\[\begin{align*}
p(E_1) = & 1 / \\
& \quad \left(1 + K_{\text{Na}} \cdot [\text{Na}]_o + K_{\text{Na}} \cdot [\text{Na}]_o \cdot K_{\text{Cl}} \cdot [\text{Cl}]_o + K_{\text{Na}} \cdot [\text{Na}]_o \cdot K_{\text{Cl}} \cdot [\text{Cl}]_o \cdot K_{\text{K}} \cdot [\text{K}]_o + K_{\text{Na}} \cdot [\text{Na}]_o \cdot K_{\text{Cl}} \cdot [\text{Cl}]_o \cdot K_{\text{K}} \cdot [\text{K}]_o \cdot K_{\text{Cl}} \cdot [\text{Cl}]_o \cdot p(E_1)\right)
\end{align*}\]

\[\begin{align*}
p(E_2) = & 1 / \\
& \quad \left(1 + K_{\text{Cl}} \cdot [\text{Cl}]_i + K_{\text{Cl}} \cdot [\text{Cl}]_i \cdot K_{\text{K}} \cdot [\text{K}]_i + K_{\text{Cl}} \cdot [\text{Cl}]_i \cdot K_{\text{K}} \cdot [\text{K}]_i \cdot K_{\text{Cl}} \cdot [\text{Cl}]_i \cdot K_{\text{K}} \cdot [\text{K}]_i \cdot K_{\text{Cl}} \cdot [\text{Cl}]_i \cdot p(E_2)\right)
\end{align*}\]

In the reduced two-state model

\[\text{[Na}^+]_i = [\text{Na}^+] \cdot \exp (-0.82 \cdot F \cdot V_m / R / T)\]
\[ J_{\text{SKCC1}} = M_{\text{SKCC1}} \cdot (k_{f\text{full}} \cdot p(E_1NaClKCl) \cdot y - k_{b\text{full}} \cdot p(E_2NaClKCl) \cdot (1 - y)) \]
\[ M_{\text{SKCC1}} = 0.029 \]
\[ \alpha_y = k_{f\text{full}} \cdot p(E_1NaClKCl) + k_{b\text{empty}} \cdot p(E_1) \]
\[ \beta_y = k_{b\text{full}} \cdot p(E_2ClKClNa) + k_{f\text{empty}} \cdot p(E_2) \]

**SR Ca\textsuperscript{2+} Pump**

\[
E_2Ca \xleftrightarrow{K_{Ca}} E_2 \quad 1 - y
\]
\[
k_1 \uparrow \downarrow k_2 \quad k_3 \uparrow k_4 \quad \beta \uparrow \downarrow \alpha
\]

\[
E_1Ca \xleftrightarrow{K_{Ca}} E_1 \quad y
\]

\[ K_dCa_i = 0.0008, \quad K_dCa_{up} = 0.08 \]
\[ k_i = 0.01, \quad k_2 = \frac{1}{0.1}, \quad k_3 = 1, \quad k_4 = 0.01 \]

\[ p(E_1Ca) = 1 / (1 + K_dCa_{up} / [Ca^{2+}]_{up}) \]
\[ p(E_1) = 1 - p(E_1Ca) \]
\[ p(E_2Ca) = 1 / (1 + K_dCa_i / [Ca^{2+}]_i) \]
\[ p(E_2) = 1 - p(E_2Ca) \]

In the reduced two-state model

\[
I_{\text{SR}} = 985 \cdot (k_2 \cdot p(E_1Ca) \cdot (1 - y) - k_1 \cdot p(E_1Ca) \cdot y)
\]
\[
\alpha_y = k_2 \cdot p(E_1Ca) + k_3 \cdot p(E_2)
\]
\[
\beta_y = k_1 \cdot p(E_1Ca) + k_4 \cdot p(E_2)
\]

**Table S11. RyR and SR Ca\textsuperscript{2+} kinetics**

<table>
<thead>
<tr>
<th>ISRRyR close open unavailable</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ k_1 = -150 \cdot i_{CaL} \cdot p(open_{CaL}) ]</td>
</tr>
<tr>
<td>[ k_2 = 0.08 / (1 + 0.36 / [Ca^{2+}]_{rel}) ]</td>
</tr>
<tr>
<td>[ k_3 = 0.000377 \cdot [Ca^{2+}]_{rel}^2, \quad k_4 = 0.000849 ]</td>
</tr>
<tr>
<td>[ I_{\text{SR RyR}} = 376 \cdot ([Ca^{2+}]<em>{rel} - [Ca^{2+}]</em>{i}) \cdot p(open_{RyR}) ]</td>
</tr>
</tbody>
</table>

**ISRT**

\[ I_{\text{ISRT}} = 2.3 \cdot ([Ca^{2+}]_{up} - [Ca^{2+}]_{rel}) \]

**ISRL**

\[ I_{\text{ISRL}} = \]
\[ I_{SR} = 5 \cdot ([\text{Ca}^{2+}]_{up} - [\text{Ca}^{2+}]_{i}) \]

**Ca^{2+} concentrations in SR**

\[ \frac{d[\text{Ca}^{2+}]_{rel}}{dt} = C_m \cdot (I_{SR}T - I_{SR}) / z_{Ca} / F / V_{rel} \]

\[ \frac{d[\text{Ca}^{2+}]_{up}}{dt} = C_m \cdot (I_{SR}U - I_{SR}T - I_{SR}L) / z_{Ca} / F / V_{up} \]

**Table S12. Contraction**

![Diagram of calcium ion concentrations](attachment:image.png)

\( \alpha_1 = 39 \text{ (mM/msec)}, \beta_1 = 0.03 \)

\[ \alpha_2 = 0.0039 \left( 0.54 \cdot \frac{K_{d}PL_i}{K_{d}PL_i + [PL]} + 0.46 \right) \]

\[ \beta_2 = 0.0039 \left( \frac{K_dATP_i}{[ATP\text{ total}]} \right)^{3/2} \]

\( \alpha_3 = 0.03, \beta_3 = 1560 \text{ (mM/msec)} \)

\[ \alpha_4 = 0.12 \left( \frac{1}{1 + \left( \frac{K_dATP_i}{[ATP\text{ total}]} \right)^3} \right), \quad \alpha_5 = 0.027 \left( \frac{1}{1 + \left( \frac{K_dATP_i}{[ATP\text{ total}]} \right)^3} \right) \]

\( K_{dPL_i} = 1.83, K_{dATP_i} = 0.1 \)

\( dX / dt = -B \cdot (h - h_i), B = 1.2, h_i = 0.005 \text{ (m)} \)

**effectiveCB = e^{20(hSMLeCaeffectiveT)}**

\[ Q_1 = \alpha_1 \cdot Ca_i \cdot p(T) - \beta_1 \cdot p(TCa) \]

\[ Q_2 = \alpha_2 \cdot p(TCa) \cdot \text{effectiveTCa} - \beta_2 \cdot p(TCa^*) \]

\[ Q_3 = \alpha_3 \cdot p(TCa^*) - \beta_3 \cdot Ca_i \cdot p(T^*) \]

\[ Q_4 = \alpha_4 \cdot p(T^*) + \alpha_5 \cdot (dX / dt)^2 \cdot p(T^*) \]

\[ Q_5 = \alpha_5 \cdot (dX / dt)^2 \cdot p(TCa^*) \]

\[ dp(TCa) = Q_1 - Q_2 \]

\[ dp(TCa^*) = Q_2 - Q_3 - Q_5 \]

\[ dp(T^*) = Q_3 - Q_4 \]

\[ p(T) = 1 - p(TCa) - p(TCa^*) - p(T^*) \]

**FoceCB = 1800000 \cdot [\text{TroponinC}] \cdot (p(TCa^*) + p(T^*)) \cdot (hSMLeCaeffectiveT) \)**
\[ F_{\text{O}2 \text{comp}} = 140000 \cdot (0.97 - h_{\text{SML}})^3 + 200 \cdot (0.97 - h_{\text{SML}}) \]

Table S13. Mitochondria

\[ v_{\text{DH}} = \frac{15000004679}{1 + \frac{100}{[\text{NADH}]}} \]

\[ v_{\text{C1}} = 15 \cdot 0.0000039825 \cdot G_{\text{C1}} \]

\[ v_{\text{C3}} = 15 \cdot 0.0000022735 \cdot G_{\text{C3}} \]

\[ v_{\text{SN}} = 15000005719 \cdot \frac{\gamma - 1}{\gamma + 1}, \gamma = 10^{\text{Gpp/2et}} \]

\[ v_{\text{EX}} = \frac{150000090953}{1 + \frac{1}{[\text{ADP}]}} \]

\[ v_{\text{PY}} = 15 \cdot 1.1570167 \cdot ([\text{P}]_{\text{ex}} - [\text{P}]_{\text{mit}}) \]

\[ [\text{P}]_{\text{ex}} - [\text{P}]_{\text{mit}} / \left(1 + 10^{\text{P}_{\text{ex}} / 48}\right) \]

\[ v_{\text{LK}} = 15 \cdot 0.000000416667 \cdot \left(e^{0.038 \cdot P} - 1\right) \]

Differential equations

\[ \frac{d[N\text{ADH}]_{\text{mit}}}{R_{\text{mc}}} = (v_{\text{DH}} - v_{\text{C1}}) / R_{\text{mc}} / 5 \]

\[ \frac{d[UQ\text{H}_2]_{\text{mit}}}{R_{\text{mc}}} = (v_{\text{C1}} - v_{\text{C3}}) / R_{\text{mc}} \]

\[ \frac{d[\text{c}^3+]_{\text{mit}}}{R_{\text{mc}}} = (v_{\text{C3}} - 2 \cdot v_{\text{C1}}) / R_{\text{mc}} \]

\[ \frac{d[H]_{\text{mit}}}{R_{\text{mc}}} = \left(2 \cdot (2 + 2 \cdot u) \cdot v_{\text{C1}} + (4 - 2 \cdot u) \cdot v_{\text{C3}} + 4 \cdot v_{\text{C1}} - 2.5 \cdot v_{\text{SN}} - u \cdot v_{\text{EX}} - (1 - u) \cdot v_{\text{PI}} - v_{\text{LK}} / R_{\text{mc}} + v_{\text{mfreemit}} / R_{\text{mc}} \right) \]

\[ \frac{d[\text{A TP}_\text{total}]_{\text{mit}}}{R_{\text{mc}}} = (v_{\text{SN}} - v_{\text{EX}}) / R_{\text{mc}} \]

\[ \frac{d[\text{P}i]_{\text{total}]_{\text{mit}}}{R_{\text{mc}}} = (v_{\text{PY}} - v_{\text{SN}}) / R_{\text{mc}} \]

Calculations

\[ [c^3+] = [c] - [c^2+], [c] = 0.27 \]

\[ [UQ] = [U] - [UQH_2], [U] = 1.35 \]

\[ [\text{NAD}] = [N] - [\text{NADH}], [N] = 2.97 \]

\[ [\text{ADP}_\text{total}]_{\text{mit}} = [\text{A}_\text{total}]_{\text{mit}} - [\text{A TP}_\text{total}]_{\text{mit}}, [\text{A}_\text{total}]_{\text{mit}} = 16.26 \]

\[ [\text{ATP}_\text{free}]_{\text{mit}} = [\text{ATP}_\text{total}]_{\text{mit}} / (1 + [\text{Mg}_\text{free}]_{\text{mit}}) / 0.017, [\text{Mg}_\text{free}]_{\text{mit}} = 0.38 \]

\[ [\text{ATP}_\text{Mg}]_{\text{mit}} = [\text{ATP}_\text{total}]_{\text{mit}} - [\text{ATP}_\text{free}]_{\text{mit}} \]

\[ [\text{ADP}_\text{free}]_{\text{mit}} = [\text{ADP}_\text{total}]_{\text{mit}} / (1 + [\text{Mg}_\text{free}]_{\text{mit}}) / 0.282 \]

\[ [\text{ADP}_\text{Mg}]_{\text{mit}} = [\text{ADP}_\text{total}]_{\text{mit}} - [\text{ADP}_\text{free}]_{\text{mit}} \]
\[ [H]^+_{\text{tot}} = 10^{\text{pH}_{\text{mit}} - 0.01] \]

\[ pH = Zett \cdot (\text{pH}_{\text{mit}} - \text{pH}), \text{pH}_i = 7.0 \]

\[ p = 1 / (1 - u) \cdot pH \]

\[ \psi = -(p - pH) \text{ (mV)} \]

\[ \psi_{\text{mit}} = 0.65 \cdot \psi \text{ (mV)}, \psi_i = -0.35 \cdot \psi \text{ (mV)} \]

\[ Zett = 2.303 \cdot 1000 \cdot R \cdot T / F \]

\[ u = \psi / p (= 0.861) \]

\[ r_{\text{buffer,mit}} = 0.022 \cdot c_{\text{at,mit}}, c_{\text{at,mit}} = (10^{\text{pH}_{\text{mit}} - 10^{\text{pH}_{\text{mit}} - 0.001}}) / 0.01 \]

\[ G_{SN} = 2.5 \cdot p - G_p \]

\[ G_p = 31.9 \cdot 1000 / F + Zett \cdot \log(1000 \cdot [\text{ATP}_{\text{total}}]_{\text{mit}} / [\text{ADP}_{\text{total}}]_{\text{mit}} / [\text{PI}_{\text{total}}]_{\text{mit}}) \]

\[ G_{CI} = E_{\text{mt}} - E_{\text{mt}} = p \cdot 4 / 2 \]

\[ G_{CJ} = E_{\text{mt}} = E_{\text{mt}} = p \cdot (4 - 2u) / 2 \]

\[ E_{\text{mt}} = E_{\text{mt}} + \text{Zett} / 2 \cdot \log ([\text{NAD}] / [\text{NADH}]), E_{\text{mt0}} = 85 \text{ (mV)} \]

\[ E_{\text{mu}} = E_{\text{mu0}} + \text{Zett} / 2 \cdot \log ([\text{UQ}] / [\text{UQH}_2]), E_{\text{mu0}} = 250 \text{ (mV)} \]

\[ A_{3/2} = 10^{E_{\text{mu0}} - E_{\text{mu0}} / Zett}, E_{\text{ma0}} = 540 \text{ (mV)} \]

\[ [a^2+] = [a_3] / (1 + A_{3/2}), [a^3+] = [a_3] - [a^2+], [a_3] = 0.135 \]

Table S14. Steady state variables

<table>
<thead>
<tr>
<th></th>
<th>Steady-state values without stimulation</th>
<th>End diastolic values during 2.5 Hz stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_{\text{Na}} ) (pA/pF)</td>
<td>-0.18</td>
<td>-8.69 \cdot 10^{-2}</td>
</tr>
<tr>
<td>( I_{\text{CaT}} ) (pA/pF)</td>
<td>-1.64 \cdot 10^{-4}</td>
<td>-1.41 \cdot 10^{-4}</td>
</tr>
<tr>
<td>( I_{\text{CaL}} ) (pA/pF)</td>
<td>-5.96 \cdot 10^{-4}</td>
<td>-6.36 \cdot 10^{-4}</td>
</tr>
<tr>
<td>( I_{\text{Kf}} ) (pA/pF)</td>
<td>0.32</td>
<td>0.11</td>
</tr>
<tr>
<td>( I_{\text{Ko}} ) (pA/pF)</td>
<td>2.81 \cdot 10^{-6}</td>
<td>1.16 \cdot 10^{-4}</td>
</tr>
<tr>
<td>( I_{\text{K}} ) (pA/pF)</td>
<td>-9.92 \cdot 10^{-9}</td>
<td>-1.59 \cdot 10^{-3}</td>
</tr>
<tr>
<td>( I_{\text{Na}} ) (pA/pF)</td>
<td>-4.24 \cdot 10^{-12}</td>
<td>-4.50 \cdot 10^{-12}</td>
</tr>
<tr>
<td>( I_{\text{NSC}} ) (pA/pF)</td>
<td>-0.27</td>
<td>-0.27</td>
</tr>
</tbody>
</table>
\[
\begin{array}{ll}
I_{Kp1} (\text{pA/pF}) & 3.69 \cdot 10^{-8} \\
I_{K(Ca)} (\text{pA/pF}) & -2.03 \cdot 10^{-5} \\
I_{KATP} (\text{pA/pF}) & 5.24 \cdot 10^{-4} \\
I_{Ca} (\text{pA/pF}) & -1.78 \cdot 10^{-2} \\
I_{CIB} (\text{pA/pF}) & -1.54 \cdot 10^{-3} \\
I_{CPTR} (\text{pA/pF}) & 0.00 \\
I_{SRCC} (\text{pA/pF}) & -1.58 \cdot 10^{-3} \\
I_{NaK} (\text{pA/pF}) & 0.16 \\
I_{NaCa} (\text{pA/pF}) & -6.60 \cdot 10^{-3} \\
I_{PMCA} (\text{pA/pF}) & 5.33 \cdot 10^{-3} \\
I_{sarU} (\text{pA/pF}) & -10.39 \\
I_{sarRyR} (\text{pA/pF}) & 0.17 \\
I_{sarT} (\text{pA/pF}) & 0.17 \\
I_{sarL} (\text{pA/pF}) & 10.22 \\
J_{SRCC1} (\text{amol/msec}) & 3.41 \cdot 10^{-3} \\
[ADP_{total}]_i (\text{mM}) & 3.23 \cdot 10^{-2} \\
[AMP]_i (\text{mM}) & 1.27 \cdot 10^{-5} \\
[PI]_i (\text{mM}) & 1.35 \\
[creatinine]_i (\text{mM}) & 10.88 \\
[phosphocreatine]_i (\text{mM}) & 14.12 \\
\end{array}
\]