Impact of Sarcoplasmic Reticulum Calcium Release on Calcium Dynamics and Action Potential Morphology in Human Atrial Myocytes: A Computational Study

Jussi T. Koivumäki*, Topi Korhonen*, Pasi Tavi*

Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

Abstract

Electrophysiological studies of the human heart face the fundamental challenge that experimental data can be acquired only from patients with underlying heart disease. Regarding human atria, there exist sizable gaps in the understanding of the functional role of cellular Ca\(^{2+}\) dynamics, which differ crucially from that of ventricular cells, in the modulation of excitation-contraction coupling. Accordingly, the objective of this study was to develop a mathematical model of the human atrial myocyte that, in addition to the sarcolemmal (SL) ion currents, accounts for the heterogeneity of intracellular Ca\(^{2+}\) dynamics emerging from a structurally detailed sarcoplasmic reticulum (SR). Based on the simulation results, our model convincingly reproduces the principal characteristics of Ca\(^{2+}\) dynamics: 1) the biphasic increment during the upstroke of the Ca\(^{2+}\) transient resulting from the delay between the peripheral and central SR Ca\(^{2+}\) release, and 2) the relative contribution of SL Ca\(^{2+}\) current and SR Ca\(^{2+}\) release to the Ca\(^{2+}\) transient. In line with experimental findings, the model also replicates the strong impact of intracellular Ca\(^{2+}\) dynamics on the shape of the action potential. The simulation results suggest that the peripheral SR Ca\(^{2+}\) release sites define the interface between Ca\(^{2+}\) and AP, whereas the central release sites are important for the fire-diffuse-fire propagation of Ca\(^{2+}\) diffusion. Furthermore, our analysis predicts that the modulation of the action potential duration due to increasing heart rate is largely mediated by changes in the intracellular Na\(^{+}\) concentration. Finally, the results indicate that the SR Ca\(^{2+}\) release is a strong modulator of AP duration and, consequently, myocyte refractoriness/excitability. We conclude that the developed model is robust and reproduces many fundamental aspects of the tight coupling between SL ion currents and intracellular Ca\(^{2+}\) signaling. Thus, the model provides a useful framework for future studies of excitation-contraction coupling in human atrial myocytes.

Introduction

In cardiac myocytes, the process triggered by the action potential (AP) and resulting in the contraction of the myocyte is commonly referred to as excitation-contraction coupling (ECC) [1]. The transient elevation of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\text{\textsubscript{i}}) that underlies the contraction is initiated by the Ca\(^{2+}\) influx from the extracellular space through the L-type calcium channels (LTCCs), which causes the release of more Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) via the SR calcium release channels (ryanodine receptors; RyRs). This mechanism is known as Ca\(^{2+}\) induced Ca\(^{2+}\) release (CICR) [2]. During one contraction cycle, the Ca\(^{2+}\) influx has to be balanced with an efflux to the same compartments, in order that Ca\(^{2+}\) does not start to accumulate and impede contraction. The majority of Ca\(^{2+}\) is re-circulated back to the SR by the SR Ca\(^{2+}\)-ATPase (SERCA), leaving a smaller fraction of Ca\(^{2+}\) to be extruded from the cell by the Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX) and plasmalemmal Ca\(^{2+}\)-ATPase (PMCA).

Whilst the same CICR mechanism initiates the transient elevation of [Ca\(^{2+}\)]\text{\textsubscript{i}}, in both ventricular and atrial myocytes, there are substantial spatiotemporal differences in the properties of the atrial and ventricular Ca\(^{2+}\) transients [3,4] due to the divergent intracellular ultrastructures. Mammalian atrial myocytes lack a prominent transverse tubular system [5], which in ventricular myocytes establishes the tight coupling of the SR to sarcolemma, enabling a Ca\(^{2+}\) release that is virtually uniform throughout the cell [6]. In atrial myocytes, however, the Ca\(^{2+}\) wave arises in the periphery (junctional-SR) and then propagates to the center of the cell, activating secondary release from the corbular (non-junctional) SR compartments [3,7,8].

Facing the complexity of a highly integrated and interdependent system, mathematical modeling has become an established complement to the experimental approach in elucidation of the mechanisms that underlie cardiac electrophysiology [9]. In human studies, the role of mathematical modeling is perhaps even more important because there are substantial limitations in the quantity and quality of the human cardiac tissue that is available for in vitro experiments. In this study, we present a model of the adult human atrial myocyte that has a spatially detailed and physiologically based formulation of the Ca\(^{2+}\) release from and uptake to the SR.
In the human heart, the contraction of atrial and ventricular muscle cells is based largely on common mechanisms. There is, however, a fundamental difference in the cellular calcium dynamics that underlie the contractile function. Here, we have developed a computational model of the human atrial cell that convincingly reproduces the experimentally observed characteristics of the electrical activity and the cyclic fluctuations of the intracellular calcium concentration. With the model, we evaluate the relative roles of the most important cellular calcium transport mechanisms and their impact on the electrical behavior of the cell. Our simulations predict that the amount of calcium released from the cellular stores during each electrical cycle crucially regulates the excitability of the human atrial cell. Furthermore, the results indicate that the cellular sodium accumulation related to faster heart rates is one of the main mechanisms driving the adaptation of cardiac electrical activity. Finally, we conclude that the presented model also provides a useful framework for future studies of human atrial cells.

Intracellular Ca\(^{2+}\) diffusion and buffering

The Ca\(^{2+}\) diffusion in the bulk cytosol and in the SR was modeled with Fick’s second law of diffusion. The components of cytosolic Ca\(^{2+}\) buffering are shown in Table 2. The effect of the mobility of Ca\(^{2+}\) buffers in the bulk cytosol was implemented as described previously [15]. The amount of SR Ca\(^{2+}\) buffer (calcineurin) was fitted based on experimental SR Ca\(^{2+}\) content [16]; see Results for details. The diffusion between the junctional and bulk cytosol was modeled as an analytically diffusion equation [17]. The accessible volume for the Ca\(^{2+}\) diffusion in the cytosol and in the SR was set to 50% of the total volume of the compartments [11]. Also the accessible area for Ca\(^{2+}\) diffusion between junctional and bulk cytosol was set to 50% of the total area between these compartments.

The diffusion coefficients are shown in Table 2. Previously, it has been shown that the effective diffusion coefficient for Ca\(^{2+}\) in the SR is 8–9 \(\mu\text{m}^2/\text{s}\) [18]. The effective diffusion coefficient is smaller than the free diffusion coefficient due to the Ca\(^{2+}\) buffering in the SR [15]. In our model, the free diffusion coefficient for Ca\(^{2+}\) in the SR is 44 \(\mu\text{m}^2/\text{s}\), which yields an effective coefficient of 8–12 \(\mu\text{m}^2/\text{s}\) in the relevant range of \([\text{Ca}^{2+}]_{\text{SR}}\) (0.3–0.6 mM).

The components of cytosolic Ca\(^{2+}\) buffering have not been characterized from atrial myocytes in the same detail as in ventricular myocytes [1]. Previously, ventricular data has been used to model the atrial Ca\(^{2+}\) buffering [11]. However, implementation of a ventricular buffering system in our model resulted in almost non-existent Ca\(^{2+}\) transients with the experimental SR Ca\(^{2+}\) content [16] (data not shown). In our model, the ‘known’ cytosolic Ca\(^{2+}\) buffers are the sarcolemmal, which is assumed to be as in ventricular myocytes based on [11], and the SERCA [19], which is fitted based on Ca\(^{2+}\) transient kinetics and SR Ca\(^{2+}\) content. The rest of the buffering, consisting most likely of troponin, calmodulin and myosin, is modeled as a single arbitrary mobile buffer, which has the mobility and \(K_d\) of calmodulin [11].
...independent K-complex features of RyR Ca2+ shown in Figure 2B, the model is still capable of reproducing limits the amount of unnecessary free variables. However, as shown in Figure 2B, the model is still capable of reproducing complex features of RyR Ca2+ release, i.e. the dependence of the release on both the intracellular and SR [Ca2+] and the adaptation of the RyR open probability’s dependence on intracellular Ca2+ [20,21].

**Sarcolemmal ion currents**

The SL ion currents were mostly formulated as in the previously published model of human atrial AP [10]. All the major modifications and novel features of the ion current submodels are described in the Text S1. Minor adjustments of parameter values are listed in Table S1.

**Results**

**Sarcolemmal ion currents and action potential characteristics**

As in other excitable cells, APs in atrial myocytes reflect the coordinated activation of several voltage-gated inward (depolarizing) and outward (repolarizing) ion channel currents (Figure S3 in the Supporting Information). The major depolarizing currents in the initial phase of the AP are the I_{Na} and I_{CaL}, and I_{NCX}, during the later phase of the AP (Figure S3 B&C&G). The I_{Na} and I_{CaL} generate large repolarizing currents in the beginning of the AP, which quickly repolarize the membrane voltage back to ~30 mV after the spike (Figure S3 A&D). Following this, the repolarization is carried mostly by I_{Kr} (Figure S3E) with very little contribution by I_{K1} and I_{Ks} (Figure S3F). Although there is a significant amount of I_{K1} present in human atrial myocytes [22], it does not contribute substantially to the action potential (Figure S3F), since it is activated at voltages below ~80 mV (see Figure S2). The PMCA creates only a very small current (Figure S3G).

As a principal validation, we compared the characteristics of the emergent AP waveform of our model to published data during 1 Hz pacing (Table S3; see also Discussion). The model reproduces the experimental values for the resting membrane potential (~77 mV), AP upstroke velocity (170 mV/ms), AP amplitude (119 mV), and AP duration (APD; APD_{90} = 11 ms, APD_{30} = 239 ms) at different stages of repolarization (see Table S3 in the Supporting Information for detailed comparison).

**Intracellular Ca2+ dynamics**

In our model, the average cytosolic Ca2+ signal has a resting concentration of 0.15 μM and an amplitude of 0.58 μM (Figure 3A) at 1 Hz pacing. The reported single exponential decay constants of the Ca2+ transient range from 92 ms to 160 ms [4,23,24] and in comparative studies between atrial and ventricular myocytes, the atrial myocytes have more rapid decays [3,4,25]. In our model, the decay constant of the Ca2+ transient is 131 ms, which is in line with data from atrial myocytes and more rapid than that reported in human ventricular myocytes [26] (Table 3). As a high-level validation of the relative contribution of Ca2+ transport mechanisms that underlie Ca2+ dynamics of the model, we simulated the effect of elevation of extracellular Ca2+ from 0.9 to 3.2 mM (data not shown). In line with experimental findings [27], the diastolic and systolic [Ca2+] increased by 52% and 88%, respectively.

Although the local Ca2+ release homogenizes the cytosolic Ca2+ signal, the Ca2+ transients at different parts of the cytosol differ from the average signal (Figure 3A). In atrial myocytes, the [Ca2+] can peak in the periphery of the cell to ~1 μM during slow pacing, whereas in the center there is only a small increase in Ca2+ concentration [28]. This spatial heterogeneity is reproduced by our model (Figure 3 A&B). Also the temporal characteristics of the simulated Ca2+ dynamics agree well with in vitro findings. The time-to-peak for Ca2+ in peripheral cytosol in atrial cells has been...
estimated to be \( \sim 20 \) ms [28] and \( 57.1 \pm 4.0 \) ms [3], and at the center of cell \( 123.7 \pm 7.8 \) ms [3]. In the model, the peripheral peak is at \( 34.8 \) ms and the central peak at \( 123.6 \) ms, in line with the experimental data.

As shown in Figure 3 C&D, the delay between the peripheral and central \( \text{Ca}^{2+} \) release in atrial myocytes yields a biphasic increment during the upstroke of the whole cell \( \text{Ca}^{2+} \) transient [7]. This phenomenon is reproduced in our model with similar upstroke dynamics (Figure 3E) as recorded in human atrial myocytes [7]. We simulated a voltage clamp experiment with a corresponding protocol and obtained the release rates with a linear fit to normalized \( \text{Ca}^{2+} \) transients.

**SR \( \text{Ca}^{2+} \) dynamics**

In human atrial myocytes, the diastolic SR \( \text{Ca}^{2+} \) content, measured as the integral of NCX current during a caffeine-induced \( \text{Ca}^{2+} \) transient, has been reported to be \( 8.3 \pm 1.2 \) amol/pF [16]. Based on the calculations of Hove-Madsen et al. [16], this corresponds to \( 50.9 \pm 7.4 \) \( \mu \text{M} \) of accessible cytosolic volume with the dimensions of our model. The SR \( \text{Ca}^{2+} \) content in our model is \( 76.2 \) \( \mu \text{M} \). However, if we use the model to reproduce the caffeine-pulse experiment [16] and calculate the integral of the generated NCX current, we get a comparable value of \( 7.5 \) amol/pF for the SR \( \text{Ca}^{2+} \) content in the model (data not shown). A possible source for the difference between the integrated NCX value and the actual SR \( \text{Ca}^{2+} \) content is the fraction of \( \text{Ca}^{2+} \) that is extruded from the cell by the PMCA, which was not considered in the experimental analysis [16].

The \( \text{Ca}^{2+} \) release from the SR generates \( 79 \pm 6 \% \) of the \( \text{Ca}^{2+} \) transient amplitude in human atrial myocytes [7] and \( 77 \% \) in our model (Figure 4D). Most of the \( \text{Ca}^{2+} \) release is generated in the junctional compartment (Figure 4 A&B). During the uptake of \( \text{Ca}^{2+} \) from the cytosol to the SR, the SERCA buffers the \( \text{Ca}^{2+} \) and generates a delay in the fluxes between cytosol to SERCA (Figure 4C) and SERCA to SR (Figure 4D). At the end of the diastolic phase there is some diffusion of \( \text{Ca}^{2+} \) in the SR, which

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**Table 1.** Cell geometry and physical parameters.

| Parameter | Definition | Value | Value [ref.] |
|-----------|------------|-------|--------------|
| \( F \)     | Faraday constant | 96478 C/mol | |
| \( R \)     | Ideal gas constant | 8314 mJ/(mol K) | |
| \( T \)     | Temperature | 306.15 K | |
| \([\text{Na}^+]_o\) | Extracellular \( \text{Na}^+ \) concentration | 130 mM | |
| \([\text{Ca}^{2+}]_o\) | Extracellular \( \text{Ca}^{2+} \) concentration | 1.8 mM | |
| \([\text{K}^+]_o\) | Extracellular \( \text{K}^+ \) concentration | 5.4 mM | |
| \( C_m \) | Cell membrane capacitance | 0.05 nF | 0.00519±0.0035 nF [10] |
| \( L_m \) | Radius of the bulk cytosol | 6.5 \( \mu \text{m} \) | 4.6±0.3 \( \mu \text{m} \) [74] |
| \( l_{cell} \) | Length of the cell | 122.051 \( \mu \text{m} \) | 88±6.1 \( \mu \text{m} \) [74] |
| \( V_{ss} \) | Volume of the subspace | 4.99232 \( 10^{-5} \) nL | |
| \( dr \) | Width of bulk cytosol compartments | 1.625 \( \mu \text{m} \) | \~2 \( \mu \text{m} \) [12,13] |

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**Table 2.** Parameters of the \( \text{Ca}^{2+} \) buffering.

| Parameter | Definition | Value [ref] |
|-----------|------------|--------------|
| \( D_{Ca} \) | diffusion coefficient for \( \text{Ca}^{2+} \) | 780 \( \mu \text{m/s} \) [76] |
| \( D_{CaBu} \) | diffusion coefficient for \( \text{Ca}^{2+}\)-buffer complex | 25 \( \mu \text{m/s} \) [11] |
| \( D_{CaSR} \) | diffusion coefficient for \( \text{Ca}^{2+} \) in SR | 44 \( \mu \text{m/s} \) [18,77] |
| \([\text{BCa}]\) | Arbitrary cytosol \( \text{Ca}^{2+} \) buffer | 0.024 mM [2] |
| \([\text{SILow}]\) | Phospholipid concentration (low-affinity sites) | 165 mM [78] |
| \([\text{SILhigh}]\) | Phospholipid concentration (high-affinity sites) | 13 mM [78] |
| \( K_{BCa} \) | Dissociation constant for arbitrary cytosol \( \text{Ca}^{2+} \) buffer | 0.00238 mM [79] |
| \( K_{SILow} \) | Dissociation constant for low-affinity phospholipid sites | 1.1 mM [80] |
| \( K_{SILhigh} \) | Dissociation constant for high-affinity phospholipid sites | 0.013 mM [80] |

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The AP appears to be shortened when the adaptation is not inhibited (left) the dependence of the RyR open probability (y axis) on the intracellular Ca\(^{2+}\) concentrations.

The time constant \(\tau\) refers to the adaptation that modulates the steady-state probabilities of open and closed gates. (B) The effect of RyR adaptation. When the adaptation is not inhibited (left) the dependence of the RyR open probability (y axis) on the intracellular Ca\(^{2+}\) adapts in line with experimental findings \([33,34]\). Inset shows the dynamics of the open and closed gate in the control situation. The 0.33-fold Ca\(^{2+}\) was implemented by reducing the maximal currents/rates of the NCX, LTCCs is too weak to trigger the CICR at the first release site in the bulk cytosol (Figure 5C, lower panel).

**Intracellular Ca\(^{2+}\) transient and action potential duration**

Above we have shown that our myocyte model can reproduce the experimentally observed impact of SR Ca\(^{2+}\) release on the inactivation of \(I_{\text{CaL}}\) (Figure S1C) and amplitude of the Ca\(^{2+}\) transient (Figure 4B). In addition, our results indicate that the SR Ca\(^{2+}\) release, especially junctional, affects the membrane voltage also via the NCX (Figure 5 B&D). To further study the effect of intracellular Ca\(^{2+}\) dynamics on AP morphology, we simulated the acute effect of total block and 3-fold increase of Ca\(^{2+}\) release (Figure 6).

The results indicate that the amplitude of the Ca\(^{2+}\) transient (Figure 6 A) has a substantial effect on the APD (Figure 6B). Blocking the SR Ca\(^{2+}\) release slows down the early phase of repolarization (Figure 6B, inset), but speeds up the late repolarization (Figure 6B), whereas the 3-fold increase of Ca\(^{2+}\) release has an opposite effect. Slowed AP repolarization causes the sodium channels to remain inactivated for a longer time. Consequently, the duration of the refractory period is increased and a prematurely applied second stimulus is unable to trigger the next AP (Figure 6D).

To further dissect the role of [Ca\(^{2+}\)]\(_i\) in AP morphology, we simulated separately the effect of decay and amplitude modulation of intracellular Ca\(^{2+}\) transients on the APD (Figure 7).

That is, the junctional [Ca\(^{2+}\)]\(_i\) was "clamped" to three different modes (Figure 7 A& E) in both cases. This modification was implemented by replacing differential variable of junctional [Ca\(^{2+}\)]\(_i\) with an analytical equation that was fitted manually to the simulated control [Ca\(^{2+}\)]\(_i\) trace. Then either the decay (Figure 7 A) or the amplitude (Figure 7 E) of the junctional Ca\(^{2+}\) transient was modified. As the results show, the accelerated decay shortens the AP substantially, whereas deceleration of decay has an opposite effect (Figure 7B).

The decay modulation has little or no effect on the early repolarization of the membrane voltage (Figure 7B, inset). The underlying mechanism appears to be the changed \(I_{\text{NCX}}\) (Figure 7C), whereas \(I_{\text{CaL}}\) is not affected (Figure 7D).

Similar to the effect of decay modulation, the amplitude of the [Ca\(^{2+}\)]\(_i\) transient affects the APD substantially (Figure 7F). However, compared to the modulation by the [Ca\(^{2+}\)]\(_i\) transient decay, increasing the amplitude also affects the early phases of repolarization (Figure 7F, inset) by enhancing the outward peak current via the NCX and accelerating the inactivation of \(I_{\text{CaL}}\) (Figure 7H). This results in a more complex modulation scheme, in which the increased [Ca\(^{2+}\)]\(_i\) transient amplitude accelerates the early repolarization and decelerates the late repolarization (Figure 7F).

**Rate dependence of action potential duration**

Increasing the pacing rate causes an immediate (within a few APs) and then a gradual (reaching steady state over several minutes) decrease in the APD of atrial myocytes; this has been shown in numerous studies. Experimental findings indicate that...
Figure 3. Ca\textsuperscript{2+} dynamics of the model. (A) The Ca\textsuperscript{2+} transients at different parts of the cytosol differ from the average cytosolic Ca\textsuperscript{2+} signal. The x-coordinates of the compartments are as follows: subspace 0.01 μm, bulk1 = 0.8325 μm, bulk2 = 2.4575 μm, bulk3 = 4.0825 μm, and bulk4 = 5.7075 μm; SL = 0 μm. (B) Spatiotemporal representation of [Ca\textsuperscript{2+}] demonstrates clearly the divergence of both the amplitude and delay of Ca\textsuperscript{2+} release in different parts of the cytosol. (C) and (D) The model reproduces the experimentally found biphasic increment during the upstroke of the global Ca\textsuperscript{2+} transient [7]. (E) Release rates of the two phases of the Ca\textsuperscript{2+} transient in experiments by Hatem et al. [7] and simulations. The two values of release rates (arrows in (C) indicate the stages of release) were obtained with a linear fit to normalized Ca\textsuperscript{2+} transients.

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Table 3. Comparison of Ca\textsuperscript{2+} characteristics of the developed model and experiments.

| Parameter                              | Simulated value | Experimental value [ref] |
|----------------------------------------|-----------------|--------------------------|
| Ca\textsubscript{aust}                | 0.146 μM        | 0.164±0.008 μM [70] T = 23°C |
| Ca\textsubscript{aust}                | 0.723 μM        | 0.251±0.039 μM; rat atrial [4] T = 23°C |
| Δ(Ca\textsuperscript{2+}) \textsubscript{ij} | 0.577 μM       | 0.185±0.039 μM; rat atrial [4] T = 23°C |
| peripheral peak 1.4 μM; center/peripheral ampl = 0.41 | ~1 μM peripheral peak and only a small rise in center; rat atrial [28] T = 22°C |
| [Ca\textsuperscript{2+}] time-to-peak peripheral/center half peak | 34.8 ms (ss), 54.8 ms (1\textsuperscript{st} bulk)/123.6 ms (last bulk) | 57.1±4.0 ms/123.7±7.8 ms; rat atrial [3] |
| Ca\textsuperscript{2+} t \textsubscript{130.8 ms} | shorter than ventricular cell [81] |
| Ca50% relax time                      | 122.9 ms        | 177.5±9.0 [27] T = 37°C |
| Ca\textsuperscript{2+} transient duration | ~600 ms         | 539.1±31.0 [27] T = 37°C |
| Ca\textsuperscript{2+} decay rate constant (1/s) | 7.28 1/s | 7.4±0.6 1/s; rat atrial [4] T = 23°C |
| SR Ca\textsuperscript{2+} content     | 76.2 μM         | 50.9±7.4 μmol/l/cytosol [16] T = 23°C |
| Ca\textsuperscript{2+} propagation speed | 79.0 μm/s⇒12.7 ms/μm (estimated from contour plot) | 91.2±12.2 μm/s; rat atrial [3] |
| peripheral peak at 34.8 ms            | ~20 ms; rat atrial [28] |

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this adaptation coincides with functional changes in the LTCC following calcium overload [29,30], and it is thus seen as one of the main mechanisms that underlie the changes in the APD [31,32]. However, in ventricular myocytes, one of the important factors in rate dependence has been shown to be the accumulation of cytosolic Na⁺ during fast pacing [33,34,35,36]. To further study this phenomenon in atrial myocytes, we simulated pacing experiments within a physiologically relevant range of frequencies or basic cycle lengths (BCLs).

To account for the other rate-dependent mechanisms that affect the APD, we implemented two additional variants of the myocyte parameters of model variant, we simulated the same protocol with each BCL, protocol results in a much steeper dependence of APD in the BCL range of [1600, 400] ms (−35%), compared to the shortening of APD (−27%) shown in Figure 8C.

Simulation results suggest that the mechanism that links AP shortening (Figure 8E) to Na⁺ accumulation is the Na⁺/K⁺-ATPase (NKA). The enhanced pumping function of NKA lead to a dramatic increase in the current (\(i_{\text{NKA}}\)) (Figure 8G). Accordingly, this mechanism has been reported previously in human atrial fibers [42] and guinea pig ventricular myocytes [43]. To confirm that this feature of the myocyte model does not depend on the Na⁺ parameters of model variant, we simulated the same protocol with model variant \(v\text{Ca} (\text{no junctional Na⁺ compartment})\) and found that fast pacing resulted in a similar increase in \(i_{\text{NKA}}\) (data not shown).

Rate-dependent changes in the NCX current

To evaluate the extent that Na⁺ accumulation due to fast pacing has on the \(Ca^{2+}\) dynamics via modulation of the NCX function, we simulated a previously used pacing protocol [44], in which the pacing frequency was increased in a stepwise manner from 1 to 2 Hz and further to 3 Hz. The results provide a general level validation for the rate dependence of \(Ca^{2+}\) dynamics of the model (Figure 9A) that correspond qualitatively to the measured force (Figure 9B).

To further study the role of NCX in the rate dependence of \(Ca^{2+}\) dynamics, we evaluated the role of the NCX as a secondary trigger of the CICR process. Figure 9E shows that whereas the \(Ca^{2+}\) influx via LTCC is decreased slightly (integral decreases by model (Figure 8C). Although the variance of absolute APD₉₀ values reported in the literature is large, the shape of the curve is similar in experiments and simulations. The relative change of APD₉₀ in the range of BCL = [1600, 400] in our model (−32%, −30% and −27% for the model variants \(v\text{Ca} , v\text{CaNass} \) and \(v\text{CaNassIk} \), respectively) fits well to the in vitro values of Boutjdir et al. [39] (−44%) and Dawodu et al. [38] (−38%). A multitude of measurement protocols are used to study the rate dependence of AP morphology in cardiac myocytes. Most importantly, the length of the period, after which the APD is determined, ranges from tens of seconds [31,40] to five minutes [41]. As the continued pacing potentially increases the \([Na^+]_o\), we wanted to study the rate dependence of AP with a second pacing protocol, in which simulation is always started from the quiescent steady-state and continued for five minutes for each BCL, separately (representative data is shown in Figure 8D). To account for all rate-dependent mechanisms described above, we performed these simulations with the \(v\text{CaNassIk} \) model variant.

Comparison of APD₉₀ after 30 seconds and 5 minutes of pacing highlights the dynamic nature of rate dependence (Figure 8E). That is, the shortening of the AP in response to faster pacing becomes more pronounced as pacing is continued. This is affected substantially by the increasing \([Na^+]_o\) (Figure 8D). If the intracellular \([Na^+]_i\) is “clamped” to the quiescent steady-state value (7.8 mM), the effect of continued fast pacing on the APD is dramatically reduced (Figure 8E, black line). Furthermore, the simulation results suggest that Na⁺ accumulation is actually a mechanism that is required for rate-dependent adaptation of APD, because the myocyte model failed to produce a normal AP at BCL 300 ms when \([Na^+]_o\) was “clamped” the quiescent steady-state value (Figure 8E, open triangle in the black trace). This failure was present already during the first few seconds of the simulation at BCL 300 ms (open diamond, in the grey trace).

Results shown in Figure 8E suggest that roughly half of the rate-dependent adaptation of AP comes from the short-term ionic mechanisms that operate in the timescale of seconds, whereas the long-term adaptation responsible for the other half of APD decrease takes minutes to develop. Furthermore, the longer pacing protocol results in a much steeper dependence of APD in the BCL range of [1600, 400] ms (−35%), compared to the shortening of APD (−27%) shown in Figure 8C.
~14% during fast pacing, the flux via reverse mode of NCX is increased considerably (integral increases over two-fold). However, since relative contribution of NCX is much smaller, we performed an additional simulation analysis to quantify its role in rate-dependent modulation of Ca\(^{2+}\) dynamics. In line with a previous study [45], blocking the reverse mode of the NCX delays the peak of [Ca\(^{2+}\)]\(_i\) by 1.8 ms (109.1 ms vs. 110.9 ms for control and block, respectively) when the pacing frequency is 1 Hz. This effect is more pronounced during fast pacing: the delay increases to 2.4 and 3.5 ms with 2 and 3 Hz, respectively. Thus, the results suggest that the rate-dependent modulation of NCX could mediate a shortening of the delay between the electrical excitation and the peak of Ca\(^{2+}\) transient.

**Discussion**

We have developed a mathematical model of the human atrial myocyte with a spatially detailed and atria-specific description of intracellular [Ca\(^{2+}\)]\(_i\) dynamics. The presented results indicate that the model accurately replicates crucial experimental findings: instead of a homogenous Ca\(^{2+}\) release, ECC is driven by a Ca\(^{2+}\) wave that arises first in the periphery and then propagates to the cell center [3,7,8]. This novel model enabled a dissective analysis of the interrelations between [Ca\(^{2+}\)]\(_i\), sarcolemmal ion currents, and SR Ca\(^{2+}\) release. The results highlight the importance of the junctional SR Ca\(^{2+}\) release sites in the ECC. The APD\(_{90}\) is shortened by 34%. Due to the lower [Ca\(^{2+}\)]\(_i\) in the junctional compartment, the depolarizing \(I_{NCX}\) is not activated to the normal level.

Figure 5. Role of the junctional vs. bulk SR Ca\(^{2+}\) release sites in the ECC. (A) Inhibition of the release sites (at t = 1 s) in the bulk cytosol has only a small impact on the ECC. The amplitude of the global Ca\(^{2+}\) transient is decreased by 31%. The Ca\(^{2+}\) signal becomes relatively inhomogeneous and it is not carried all to the center of the cell. (B) The abnormal Ca\(^{2+}\) dynamics also affect the AP: APD\(_{90}\) is shortened by 10%. Since there is less Ca\(^{2+}\) to be extruded, the \(I_{NCX}\) is reduced. (C) Inhibition of the junctional SR Ca\(^{2+}\) release site (at t = 1 s) has a profound effect on the ECC. The amplitude of the global Ca\(^{2+}\) transient is decreased by 58%. Without the junctional SR Ca\(^{2+}\) release, the Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channels is too weak to trigger the CICR at the first release site in the bulk cytosol. Thus, the inhibition of the junctional release results in a failure in the propagation of the Ca\(^{2+}\) signal. (D) The APD\(_{90}\) is shortened by 34%. Due to the lower [Ca\(^{2+}\)]\(_i\) in the junctional compartment, the depolarizing \(I_{NCX}\) is not activated to the normal level.

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Principal characteristics of the model and relevance to previous in silico studies

It is well known that in the human atria the cellular AP shape varies depending, for example, on gender, age and personal ongoing medication plan. In addition to the physiological variation, studies using human tissue samples are always affected by the parallel pathophysiology of the patients who are undergoing surgery due to some other cardiac malfunction. Experimentally, the morphology of the human atrial AP ranges from a triangular AP shape with no sustained plateau to a long AP with a spike-and-dome shape [46,47,48]. The AP heterogeneity has been shown to correlate tightly with the relative expression levels of ion channels in different regions of the atria in an experimental canine model [49] and in human patients [50]. In the model presented here, the AP characteristics (resting membrane potential, upstroke velocity, amplitude and duration) emerging from an accurate description of individual ion currents are well within the range of experimental data reported in the literature.

During the last decade, mathematical modeling has become an established complement to experimental work in attempts to elucidate the ionic mechanisms that underlie the electrophysiology of cardiac myocytes [9]. In the case of human atrial myocytes models, the platform was established by the individual works of Nygren et al. [10] and Courtemanche et al. [31]. The usability of these comprehensive frameworks has been established, e.g. in consecutive in silico studies of AP morphology [37,51] and atrial fibrillation [52,53,54]. While these models provide a detailed description of the transmembrane ion currents, very little emphasis has been placed on the accurate description of the spatiotemporal properties of intracellular Ca2+. Experimental findings indicate that there are substantial spatiotemporal differences in the properties of the atrial and ventricular Ca2+ transients [3,4], thus we have developed a novel model that considers the atrial-specific properties of Ca2+ signaling.

Ca2+ dynamics of the model

In contrast to the virtually uniform Ca2+ release in ventricular myocytes [6], it is characteristic for atrial myocytes that the Ca2+ wave, initiated by the CICR mechanism, arises first in the periphery and then propagates to the cell center [3,7,8]. Consequently, the delay between the peripheral and central Ca2+ release in atrial myocytes yields a biphasic increment during the upstroke of the Ca2+ transient [7]; a phenomenon that is reproduced in our model with accurate spatiotemporal parameters. This delay can be decreased by inotropic interventions that promote increased SR Ca2+ content, and consequently enhanced SR Ca2+ release; thus, it establishes a mechanism through which...
Figure 8. Role of Na+ accumulation in the rate dependence of the AP. The myocyte was paced as previously [38] with a stepwise reduction in BCL, and corresponding AP characteristics were defined for each BCL. (A) Shortening of the BCL increases both the subsarcolemmal and cytosolic [Na+], especially at BCLs 400 ms and 300 ms. (B) The values of APD90 calculated from simulations are compared to those reported by Dawodu et al. [38], and Neef et al. [70]. (C) The steep dependence of APD90 on the BCL reported by Dawodu et al. [38], Bosch et al. [72] and Dobrev & Ravens [73] is reproduced by the model. (D) Continued fast pacing starting from a quiescent steady-state results in a dramatic accumulation of intracellular Na+. (E) APD adaptation in control situation conditions and [Na+]i “clamped” to the quiescent steady-state value. (F) AP shape at BCLs 1600, 800 and 400 ms after 5 minutes of pacing starting from a quiescent steady-state. (G) Accumulation of Na+ due to fast pacing, continued for 5 minutes, increases Na+/K+-ATPase current ($I_{\text{Na+K}}$) substantially.

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the interval between the electrical excitation and the peak of Ca\(^{2+}\) transient can be modulated. Our results highlight the crucial role of the junctional SR in mediating the CICR, while the inhibition of non-junctional Ca\(^{2+}\) release sites causes only an attenuation of the Ca\(^{2+}\) signal during fire-diffuse-fire propagation. Furthermore, junctional SR Ca\(^{2+}\) release sites appear to define the interface between Ca\(^{2+}\) and AP, i.e., decreasing the junctional Ca\(^{2+}\) release shortens the AP substantially. These findings are in line with the previously suggested role of the non-junctional SR as an inotropic release reserve that can be recruited when greater contractility is required [13].

Cumulative evidence suggests that changes in the [Ca\(^{2+}\)]\(_i\) homeostasis may initiate electrical remodeling during atrial fibrillation, which is characterized by a marked shortening of the action potential plateau phase [55,56,57]. In future studies, the presented model, which is based on an atrial-specific description of Ca\(^{2+}\) signaling, has thus great potential in elucidating the function of the remodeled cells with altered Ca\(^{2+}\) homeostasis and SL ion currents.

**Impact of Ca\(^{2+}\) transient on AP morphology and cellular excitability**

The tight coupling of SL ion currents, which underlie the AP shape, and the [Ca\(^{2+}\)]\(_i\), has been well established in experimental setups of both ventricular [33,58,59] and atrial myocytes [48], as well as in computational studies [36,60]. Our results suggest that both amplitude and decay modulation of the Ca\(^{2+}\) transient produce significant changes to the APD compared to the control situation. Changes in [Ca\(^{2+}\)]\(_i\) in the vicinity of the SL affects both \(I_{\text{NCX}}\) and \(I_{\text{CaL}}\). However, while both increased amplitude and decelerated decay of the Ca\(^{2+}\) transient enhance the inward \(I_{\text{NCX}}\), the inactivation of \(I_{\text{CaL}}\) is affected only by the former. These findings indicate that in human atrial myocytes the NCX is more important than the LTCC in linking the amount of Ca\(^{2+}\) released from the SR to changes in the APD. This scheme also concurs with a previously reported role of NCX as the main mediator of the inotropic effect of AP prolongation in canine atrial myocytes [61]. Understanding the role of this mechanism is of great importance because it not only links inotropic interventions (increased amplitude of the Ca\(^{2+}\) transient) but also situations such as hypothyroidism (slowed decay of the Ca\(^{2+}\) transient) to APD modulations.

When the effects of amplitude and decay modulation of the Ca\(^{2+}\) transient are combined to a strongly modulated physiological frameset of blocked or increased SR Ca\(^{2+}\) release, the resulting changes in AP morphology are even more pronounced. That is, decreasing the release decelerates the early repolarization and accelerates the late repolarization of the membrane voltage, whereas increased SR Ca\(^{2+}\) release promotes a faster early repolarization and a slower late repolarization of the AP. Most interestingly, the increased SR Ca\(^{2+}\) release enhances the refractoriness of the AP. That is, the decelerated repolarization of the AP (due to increased \(I_{\text{NCX}}\)) slows down the recovery from inactivation of the sodium current and thus a premature electrical stimulus is unable to trigger a second AP.

**Rate-dependent modulation of action potential**

The primary physiological context, in which the duration of the AP is modulated, relates to changes in heart rate [62]. Numerous parameters of the *in situ* heart are affected simultaneously in that dynamic process, but in single cell preparations the situation is
significantly simpler. In ventricular myocytes, one of the main mechanisms that has been shown to underlie the rate dependence of the AP is the accumulation of cytosolic Na⁺ during fast pacing [33,34,35,36]. That is, as the pacing rate increases, the Na⁺ influx per unit time is increased. Since this change is not fully compensated with increased eflux, [Na⁺] increases with continued fast pacing.

As previously demonstrated in ventricular myocytes [35], this mechanism emerges from the interplay of NKA and NCX. If only NKA or NCX senses the accumulated [Na⁺], in response to continued fast pacing, the APD is decreased less compared to the normal situation, in which both of them sense an increased [Na⁺]. A similar phenomenon is apparent in our atrial model: the mere increase in pacing frequency (or reduction in BCL) does not produce a maximal shortening of the AP. Rather, the increase of [Na⁺], and the consequent change in the NKA function, caused by the continued high frequency pacing, promote a substantial further decrease of APD. Furthermore, the results indicate that the other ionic determinants of APD, emerging from the rate dependence of I_m [37] and Ca²⁺ dynamics, are effective already within a shorter time-scale (~10 s) of adaptation compared to that of Na⁺ (~minutes). Thus, our findings underline the importance of considering the effect of Na⁺ accumulation in both in vitro and in silico studies of AP rate dependence when results obtained with different protocols are interpreted or compared.

In myocytes with long AP, the increase of Ca²⁺ transient shortens the AP (via inactivation I_CaL). Interestingly, our simulation results indicate that, in this respect, the human atrial myocytes behave similar to animal with short AP. That is, increased Ca²⁺ transient amplitude (due to e.g. enhanced SR Ca²⁺ release) promotes an inward I_NCX, thus lengthening the AP.

Ca²⁺ entry through the NCX and its role in CICR

The role of the NCX as a trigger of SR Ca²⁺ release has been studied extensively in ventricular myocytes during recent years; the findings, however, are controversial [60]. While some results indicate that Ca²⁺ entry via the reverse mode of NCX is significant in physiological conditions [63,64], other studies suggest it to be important only in pathological situations [1,65].

Our simulation results suggest that in atrial myocytes the contribution of NCX to the Ca²⁺ influx is increasingly significant at higher pacing rates, even though the bulk of the flux goes via the LTCC. However, when the temporal role of these two mechanisms is compared, it is apparent that during the first few milliseconds of the CICR process the NCX contributes rather equally to the Ca²⁺ influx. Accordingly, the acute inhibition of the reverse mode of NCX delays the peak of [Ca²⁺], by a few milliseconds, depending on the pacing rate. These findings are in line with the reported delay of the Ca²⁺ transient if the Ca²⁺ entry via the NCX is inhibited in ventricular myocytes [45]. Our results thus indicate that the NCX could have an important role in accelerating the rise of [Ca²⁺], with increasing heart rate, even though LTCC is the primary trigger of CICR, as suggested previously [66]. Hence, the modulation of the NCX could promote a rate-dependent reduction of the electro-mechanical interval in the development of contractile force. The in vitro significance of the delay modulation by the NCX presents an interesting question for future simulation studies in tissue and/or whole-atria models.

Potential limitations

Variability of experimental results imposes a fundamental challenge for modeling studies that utilize data measured from isolated human atrial tissues or cells. It would thus be an irrelevant and futile effort to try to fit the electrophysiological characteristics of the model perfectly to one single set of in vitro data. Instead, it is more essential that the simulation results agree qualitatively and semiquantitatively with the majority of the measured results, which has been established for the AP morphology in our model. There is a further challenge for validation of the Ca²⁺ dynamics due to incomplete experimental data. To compensate for this, we have also compared the model behavior to ventricular data and measurements obtained with atrial animal models.

The developed RyR module of the myocyte model is an approximate description of macroscopic SR Ca²⁺ release, sharing the general limitations ‘common pool’ models. Thus, the justification for most of the chosen parameter values cannot be derived directly from the biophysical properties of RyR channels [67,68]. Instead, the ad hoc parameter values of the RyR modules are based on indirect fitting: the dynamics of intracellular Ca²⁺ were adjusted to be in line with macroscopic experimental observations [3,7,8]. The simple structure makes the RyR module computationally efficient, while still being capable of reproducing essential features of RyR Ca²⁺ release characterize the emergent properties of atrial Ca²⁺ dynamics: biphasity of the increasing Ca²⁺ concentration during the Ca²⁺ transient, and the relative contribution of SL and SR Ca²⁺ fluxes to the Ca²⁺ transient. A more complex RyR module might be needed in future studies if the simulations are run beyond the conditions that were investigated in this study.

Although our model can reproduce rather accurately the rate-dependent changes in ion dynamics, it should be noted that the description of the underlying mechanisms is by no means exhaustive. For example, one potential regulatory pathway is the Ca²⁺/calmodulin-dependent protein kinase (CaMKII) that has been studied extensively in ventricular myocytes [69]. Recent findings indicate that CaMKII could, for example, have an important role in the regulation of RyR [70] in atrial myocytes. As more experimental data become available, the effects of CaMKII on its phosphorylation targets should be considered in future modeling studies.

We have chosen to use Hodgkin-Huxley formalism for the ion currents. We acknowledge that Markovian models would allow for a more detailed description of the complex kinetics of processes (activation, deactivation, inactivation, and recovery from inactivation) that the channels exhibit. However, it can be very difficult to meet the information requirements for defining the transitional rate constants [71]. Furthermore, Markovian models can be computationally expensive compared to Hodgkin-Huxley formalism [71].

Conclusion

We conclude that the novel myocyte model provides significant insight into the excitation and ion dynamics of human atria. Our results underline the tight coupling of AP morphology to SR Ca²⁺ release and intracellular Na⁺ that are subject to strong modulation under physiological conditions. Furthermore, with a physiologically accurate description of intracellular Ca²⁺ dynamics the model is a potential tool, for example, in the elucidation of mechanisms that link changes in Ca²⁺ homeostasis to pathophysiological conditions. Thus, it offers attractive possibilities to study the electrical remodeling during atrial fibrillation, and to find potential targets that affect the refractoriness of the AP. Finally, as the computational cost of the model is low, it is also a feasible component for multi-scale models of tissue and/or heart.
Supporting Information

Figure S1 The L-type Ca\(^{2+}\) current (I\(_{\text{CaL}}\)) characteristics. (A) The time-constant for gate \(f_1\) was fitted to experimental data [1]. In contrast to the original formulation of Nygren et al. [2] (black line), the time-constant was adjusted to have larger values in the membrane voltage range of \(-40\) and \(-10\) mV (blue line); an approach that has been used previously [3] (red line). (B) The steady-state curve for the Ca\(^{2+}\) dependent inactivation gate is shown as a function of \([\text{Ca}^{2+}]\) in the junctional subspace. As the lower panel shows, this formulation reproduces qualitatively the result that blocking of SR Ca\(^{2+}\) release decreases the rate of LTCC inactivation significantly in human atrial myocytes [5]. (C) The traces show the I\(_{\text{CaL}}\) that was recorded in voltage clamp simulation; holding potential \(-80\) mV. Found at: doi:10.1371/journal.pcbi.1001067.s001 (0.14 MB TIF)

Figure S2 Steady-state activation and time-constant of I\(_r\). Experimental conditions were replicated from [12]. The current traces show the I\(_{\text{CaL}}\) that was recorded in voltage clamp in 10 mV steps, the holding potential was \(-40\) mV. The I\(_r\) model was fitted to the left atrium wall data from [12]. Found at: doi:10.1371/journal.pcbi.1001067.s002 (0.07 MB TIF)

Figure S3 The principal outputs of the myocyte model at 1 Hz pacing. (A) The model reproduces an AP that is characteristic for human atrial myocytes: a large initial peak with a narrow early plateau is followed by a late, low amplitude, plateau phase; a so-called triangular shape. (B) and (C) The major depolarizing currents in the initial phase of the AP are the I\(_{\text{Na}}\) and I\(_{\text{CaL}}\). (D) The I\(_{\text{Na}}\) (black line) and I\(_{\text{Ks}}\) (grey line) generate large repolarizing currents in the beginning of the AP. (E) The late repolarization is carried mostly by I\(_{\text{Kf}}\). (F) As demonstrated by the time courses of I\(_{\text{f}}\) (black solid line), I\(_{\text{CaL}}\) (black dashed line), and I\(_{\text{Ks}}\) (grey line), they contribute very little to the AP, compared to I\(_{\text{Na}}\) and I\(_{\text{Ks}}\). (G) During the late phase of the AP, the I\(_{\text{SCN}}\) (grey line) generates a significant depolarizing current, while the amplitude of I\(_{\text{PMCA}}\) (black line) is much smaller. Found at: doi:10.1371/journal.pcbi.1001067.s003 (0.17 MB TIF)

Table S1 Modified parameter values of the previously published model components.

Table S2 Parameters of the novel RyR model.

Table S3 Comparison of AP characteristics of the developed model (\(v\text{Ca}\)) and the two extended variants (\(v\text{CaNass}\) and \(v\text{CaNassI}\)) to experiments.

Table S4 Initial values for the differential variables at 1 Hz pacing steady-state.

Text S1 Model implementation.

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Author Contributions

Conceived and designed the experiments: JTK TK PT. Performed the experiments: JTK TK PT. Analyzed the data: JTK TK PT. Contributed reagents/materials/analysis tools: JTK TK PT. Wrote the paper: JTK TK PT.
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