Quantification of Local Calcium Releases Contribution to Diastolic Depolarization in a 3D Model of Single Rabbit Sinoatrial Node Cell

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Abstract

This work aims at investigating the role of local calcium releases (LCRs) in pacemaking. This is achieved by developing a 3D model of a single rabbit sinoatrial node cell featuring detailed membrane and calcium-clock descriptions. Secondly, single cell simulations where LCRs were prevented during the diastolic period are run to assess their effect on the cell's cycle length. Finally, two heterogeneous model cells were coupled together to study the influence of LCRs on the synchronization process. The simulations show that this model reproduces both experimental and previous models' basal rabbit sinoatrial cell action potential characteristics. Comparing simulations in control conditions with simulations where LCRs are prevented below a membrane voltage of -30 mV shows that their effect depends on the parameter randomization of heterogeneous cells. Coupled cells simulations at different gap junctional resistance values show that, below a specific inter-cellular coupling value ($R_{gap} < 10^4 M\Omega$), spontaneous cells drive dormant ones regardless of the presence of LCRs. The latters hamper the driving process at lower coupling strengths ($R_{gap} = 10^4 M\Omega$), but provide larger heart rate dynamics. Overall, this work provides a useful tool to study pacemaking and gives quantitative hints on its mechanisms.

1. Introduction

Sinoatrial node (SAN) physiology has been extensively investigated since the discovery of this fundamental cardiac tissue. Especially for small mammals (e.g., mice, rabbits, guinea pigs), lots of data have been accumulated and allowed the understanding of the role and functioning of the SAN. Computational models have also greatly contributed in elucidating the mechanisms that determine the heartbeat. However, a deep and comprehensive description of cardiac pacemaking is lacking, and in particular the quantitative role of different actors involved is still not consolidated, limiting the possibility of acting pharmacologically in case of disease.

In the SAN, intracellular calcium cycling does not have a role in contraction, but participates to homeostasis and electrophysiology through sodium-calcium exchanger (NCX) activity. In particular, stochastic openings of ryanodyne receptors (RyRs) determine local calcium releases (LCRs) from the sarcoplasmic reticulum (SR). The increased local Ca^{2+} concentration in the cytosol activates NCX which, extruding 1 Ca^{2+} ion and intruding 3 Na^{+} ions, generates a depolarizing current. While experimental evidence [1] has questioned the spontaneity of RyRs opening (and therefore the presence of an independent "calcium-clock"), this process remains crucial for AP onset [2].

Consequently, in order to provide additional quantitative information about the contribution of LCRs to diastolic depolarization, this work aims at: 1) developing a detailed, 3-dimensional single SAN cell rabbit model combining state-of-the-art membrane and calcium-clock descriptions; 2) comparing AP features of the updated model in control conditions and when LCRs are disabled and 3) investigate the contribution of LCRs to the synchronization process between 2 model cells.

2. Methods

Membrane current formulations were taken from the SDiF model [3]. The main model development in this work is represented by the adoption of the 3D detailed intracellular calcium handling description of the recent Maltsev et al. model [4]. The parameters P_{CaT} (maximum permeability of T-type calcium current) and Km_{fCa} (dissociation constant of Ca^{2+}-dependent I_{CaL} inactivation) were scaled by a factor 0.8 and 6, respectively, compared to the original SDiF model. All other cell parameters (dimension, compartmentalization, etc) were the same as in [4].

Different types of simulations were run with n = 5 heterogeneous cells, obtained by randomization of the maximal conductances, currents and permeabilities (P_{CaL}, P_{CaT}, g_{Kr}, K_{NaCa}, i_{NaKmax}, g_{Na}, g_{Ks}, g_f, g_{to}, P_{up}) via log-normal distribution ($\sigma = 0.2$) sampling [5]. In the first set of simulations, baseline conditions

were tested (20 s to reach steady-state). Secondly, 1 s simulations where LCRs were disabled during diastole or not, were performed. When LCRs were disabled, CRUs were allowed to open only when the cell membrane voltage (V_m) reached a threshold of -30 mV (higher than the take-off potential). These simulations started from the last MDP of the baseline simulation. Thirdly, 1 s coupled cells simulations with and without LCRs. Coupling was obtained by adding the gap junctional current at different R_{gap} values ($10^1, 10^2, 10^3, 10^4, \infty$ MΩ) to the cells' current balance, as previously done [5]. One cell was made "dormant" (i.e., it did not show spontaneous depolarizations) by reducing I_f maximal conductance and I_{CaT} maximal permeability to 10% of their original values. The presence of a dormant cell and the disabling of LCRs were implemented to study the effect of LCRs on inter-cellular synchronization. Steady-state conditions at 20 s and $R_{gap} = \infty$ MΩ were used as initial conditions.

To gain quantitative information in the relative contribution to diastolic depolarization, the charges transported by I_f, I_{NaCa} and I_{tot} were computed as the integral of the respective currents during diastole (from the first maximum diastolic potentail, MDP, to the first take-off potential, TOP). The first diastolic depolarization duration (DD) was computed as the time difference between the occurrence of the first MDP and the first TOP. Other AP features were computed as in [5].

The model was developed in MATLAB R2021a and integrated using an explicit Euler scheme (step = 7.5 µs) together with first order finite differences. 1s of simulation took approximately 20 minutes on an Intel(R) Core(TM) i7-8565U CPU @1.80GHz machine with 8 GB of RAM.

3. Results

The present model reproduced rabbit SAN cell physiology by showing AP features similar to previous models and compatible with experimental values (Table 1). Another important experimental validation is the simulation of funny current block by ivabradine: 66% I_f block led to a -19% rate reduction (CL = 470 ± 4 ms, + 24%). These results are similar to the SDiF model and experimental data [3]. Figure 1 shows the model APs and the calcium concentration in the subspace at (1) the beginning of the late diastolic phase; (2) the AP onset and (3) during the AP. As shown, LCRs appear in late diastole, grow in number before the AP upstroke (contributing to the exponential voltage rise) and finally merge into a global AP-induced calcium transient.

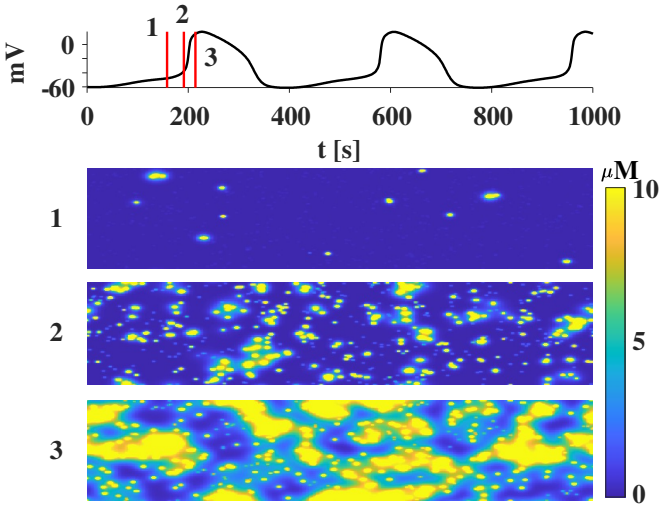

Figure 1. Action potential (top) and calcium concentration in the subspace (bottom three panels) in a steady-state (20 s) baseline simulation. Red vertical lines indicate the time at which the calcium concentration is plotted.

3.1. Disabling LCRs

Figure 2 shows the results of disabling LCRs during the diastolic depolarization of a single cell. As explained in section 2, in this simulation CRUs could open only when the membrane voltage was above -30 mV, a value at

Table 1. Comparison between models and experimental AP features. Cycle length (CL), Maximum Diastolic Potential (MDP), Action Potential Amplitude (APA), Overshoot (OS), Action Potential Duration at 50% repolarization (APD_{50}), Maximum Upstroke Velocity (dV/dt_{max}). Data is shown as mean ± standard deviation for the present model (values averaged on last 2s of 20s baseline simulations) and as mean±SD (range) for experimental values.

Feature	Present model	SDiF model [3]	Experimental values [3]
CL(ms)	379±1	352	325±42 (247÷389)
MDP(mV)	-61±0.1	-58	-56±6 (-66÷-52)
APA(mV)	78.4±0.8	80	87±6 (78÷98)
OS(mV)	19±0.7	22	27±5 (20÷32)
APD_{50}(ms)	131±0.4	108	93±12 (73÷111)
dV/dt_{max}(V/s)	6.4±0.2	7.1	11.3±6.5 (4.8÷27)

which I_{CaL} activation threshold is already reached and the AP is triggered. In this condition, the first diastolic period lasted 308 ms, representing an average +60% increase compared to baseline (193 ms).

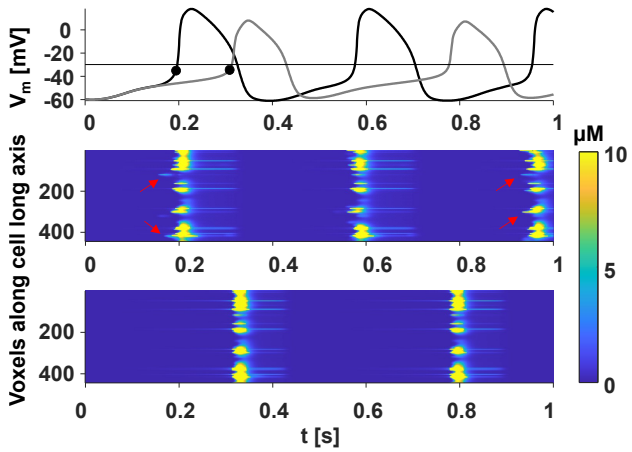

Figure 2. Comparison between AP traces (top panel) of baseline (black) and no LCRs (gray) conditions in the present model. Black dots indicate the TOP, the horizontal black line is the -30 mV LCRs activation threshold. Linescan along the cell long axis in control conditions (middle panel) and when LCRs are disabled during diastole (bottom) are reported, with red arrows marking LCRs.

In comparison, preventing Ca^{2+} release below −30 mV in the original SDiF model leads to a milder DD prolongation (243 vs 179 ms in control, +36%). Of note, the AP occurrence in the present model is delayed due to the fact that the diastolic depolarization slope in the late exponential phase is lower than in control conditions. This is a direct consequence of the reduced NCX (Table 2) caused by the absence of LCRs (compare Figure 2 middle and bottom panels), which however seems not to affect the AP firing threshold (TOP = −34.9 mV in control vs −34.2 mV when LCRs are disabled). However, the analysis on 5 heterogeneous cells provides different results: the first diastolic period lasted 166 ± 14 ms in the absence of LCRs, representing an average −15 ± 76% decrease compared to baseline (181 ± 68 ms, p>0.68). This happens despite the average reduction in Q_{NaCa} (Table 2, p = 0.02 for Q_{NaCa}/Q_{tot}). Also, the TOPs are significantly depolarized: −31.3 mV with respect to −34.2 mV in baseline (p = 0.024).

3.2. Coupled cells with and without LCRs

Figure 3 reports the results of the coupled cells simulations at different coupling values in the presence

Table 2. Comparison of charge influx ratio during first diastole in baseline and no LCRs conditions. Q_{NaCa}, sodium-calcium exchanger charge; Q_f, funny current charge; Q_{tot}, total charge.

Condition	DD (ms)	Q_{NaCa}/Q_{tot}	Q_{NaCa}/Q_f	Q_f/q_{tot}
Baseline	193	111%	87.2%	127.3%
	181±68	99.2±13%	80.6±46.4%	145±51.9%
No LCRs	308	111.3%	55%	202.3%
	166±14	56.6±14.6%	39±10.3%	145.4±9.3%

or absence of LCRs in diastole. If $R_{gap} < 10^3$ MΩ, the spontaneous cell is able to depolarize the dormant one whether LCRs are allowed or not. For $R_{gap} = 10^4$ MΩ however, in the presence of LCRs only 1 dormant cell is effectively driven to fire an AP. When LCRs are prevented, this number grows to 4, even if the DDs are prolonged (average +19.4% for the spontaneous cell and +32.4% for the driven one).

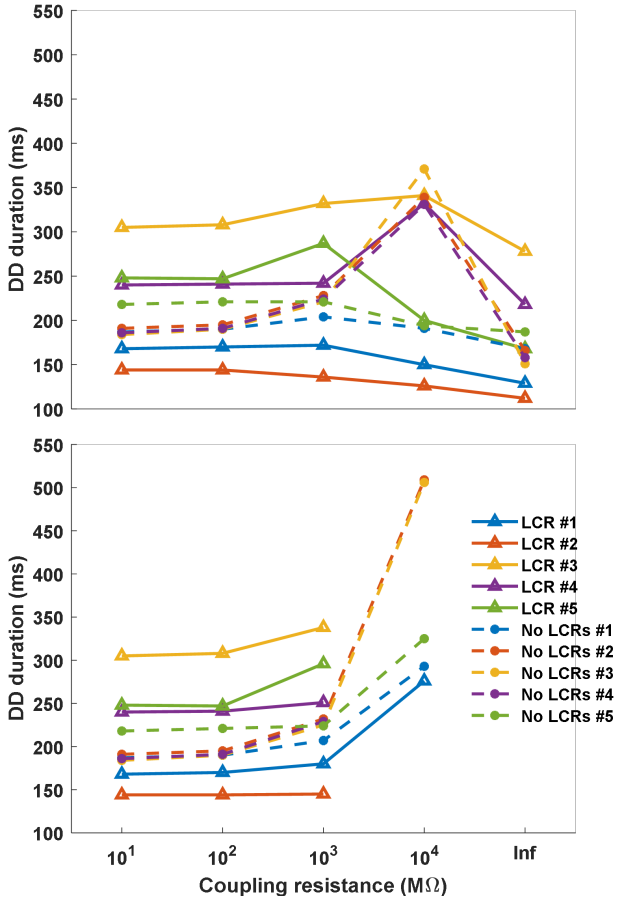

Figure 3. Diastolic depolarization durations of coupled cells (top: spontaneous cell; bottom: dormant cell) with or without LCRs during diastole at different coupling values.

Interestingly, the DD duration range is enlarged by the presence of LCRs (e.g., $196\,\mathrm{ms}$ vs $24\,\mathrm{ms}$ without LCRs and with $R_{gap} = 10^3$ MΩ, $+717\%$) when $R_{gap} < 10^4$ MΩ, coupling values at which all pairs of heterogeneous cells are completely synchronized.

4. Discussion and Conclusion

In this work, we merged detailed calcium [4] and membrane clock [3] formulations to develop a tool for future investigations, which we also started to address here. First of all, the model showed a physiological behaviour for a single rabbit sinoatrial node cell in terms of AP features values (Table 1).

Simulations in which CRUs are prevented from opening during the diastolic phase (but can release Ca^{2+} when the cell membrane reaches $-30\,\mathrm{mV}$, in order to produce the AP-induced calcium transient) show that LCRs are important in determining the late diastolic phase. If they are absent indeed, the action potential is substantially delayed because of the reduction in positive charge influx by the sodium-calcium exchanger. Interestingly, this effect is more pronounced in the present 3D model ($+60\%$ acute diastole prolongation) than in the original SDiF ($+34\%$). When considering 5 heterogeneous model cells however, variable results – with an average DD shortening ($-15 \pm 76\%$) following LCRs deactivation – are obtained despite the general reduction in the I_{NaCa} contribution in diastole (Table 2). This may suggests that LCRs contribution is dependent on the balance between Ca^{2+} and membrane clocks achieved by the parameter randomization in both the membrane (e.g., g_f and P_{CaL}) and calcium-clock (P_{up}, SERCA uptake rate).

Concerning the coupled cell simulations (Figure 3), it can be noted that in the critical condition of extremely low coupling ($R = 10^4$ MΩ), the LCRs presence reduces the chances of the spontaneous cell to drive the dormant one. At the same time however, the interaction between the heterogeneized Ca^{2+} (when LCRs are allowed to happen) and membrane clocks allows to achieve larger beating rate dynamics. This may support the idea that the membrane clock acts as a limit-cycle oscillator, providing robustness to pacemaking, while the calcium clock, by acting through criticality mechanisms, adds flexibility and consequently helps to achieve the range of physiological heart rates seen in mammals *in vivo* [6].

A limitation of the model is represented by the slightly prolonged APD_{50} values, which provide a CL at the upper limit of experimental values. However, further possible model developments - in particular regarding the membrane clock description - such as the inclusion of small-conductance calcium-activated potassium channels (I_{sK}) [7], may resolve this issue by shortening the AP, consequently allowing higher beating rates. Additionally, 100% I_f block leads to the cessation of spontaneous beating and to model instability, highlighting the possibility of improvement in the description of the fine current balance during early diastole. Also, the mechanisms behind the behaviour of LCRs in determining a lengthening or a shortening of the diastolic period must be further inspected.

In conclusion, this model can represent a valuable tool to investigate pacemaking mechanisms with a high detail of membrane and calcium clock dynamics. Preliminary evidence here reported would suggest that calcium releases are important for late diastolic depolarization and may support the idea that the calcium clock adds flexibility but cuts robustness of pacemaking also in coupled cells.

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