Extracellular Calcium and L-Type Calcium Current Inactivation Mechanisms: a Computational Study

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Abstract

Extracellular calcium concentration ($[Ca^{2+}]_o$) affects cardiac action potential (AP): their inverse dependence has already been assessed in vivo and in vitro.

Both shortening and prolongation of AP are associated with an increased risk of arrhythmias and Ca^{2+} variations may occur in many different contexts (e.g. pathological hypo/hypercalcemia, haemodialysis therapy, bed-rest experiments).

Computational modeling could provide a useful support to investigate this phenomenon: however, $[Ca^{2+}]_o$ dependence is not reproduced properly by most of the commonly used human AP models

The aim of this study has been to modify one of the most recent human ventricular cell model in order to improve its response to $[Ca^{2+}]_o$ changes.

The modified model has been validated against the same experimental data used for the original one, in order to verify its consistency, and it can thus be used to explore "in silico" the effects of electrolyte unbalances on the electrical activity of human cardiomyocytes.

1. Introduction

It is well know that extracellular calcium concentration ($[Ca^{2+}]_o$) affects cardiac action potential (AP): in fact, an increase of $[Ca^{2+}]_o$ shortens AP while $[Ca^{2+}]_o$ decrease lengthens it, as observed in different species [1–5] and human atrial cells [6]. Although experimental data on the effects of hypo- and hyper-calcaemia on human ventricular APs are not available, consistency between APD changes and corrected QT interval (QTc) can be considered [7].

Since APD variations may lead to arrhythmia onsets, $[Ca^{2+}]_o$ dependency of repolarization is very important and should be considered in all clinical contexts where electrolyte changes occur, e.g. haemodialysis sessions or bed-rest experiments.

From earlier studies [8], L-type Ca^{2+} current (I_{CaL}) seems the one mostly responsible for the APD-$[Ca^{2+}]_o$ dependence, even if there are many other ionic mechanisms involved and this phenomenon is still not completely understood.

Computational modeling can constitute a useful tool to approach and investigate these complex interactions: unfortunately, most of the published human AP models have not taken into account this dependence and therefore respond in an opposite way to $[Ca^{2+}]_o$ variations, i.e. $[Ca^{2+}]_o$ increase lengthens APD, and viceversa.

The aim of this study has been to modify the most recent human ventricular AP model (O'Hara-Rudy, [9]) in order to improve its APD-$[Ca^{2+}]_o$ dependence without altering model behaviour in control condition, as similarly done for older models in previous works [6,8].

The proper dependence should be achieved by acting mainly on I_{CaL} formulation, strengthening the Ca^{2+}-dependent inactivation (CDI) with respect to the V-dependent one (VDI).

2. Methods

The O'Hara-Rudy model of human ventricular myocyte (ORd, [9]) was used as basis. However, the L-Type Ca^{2+} current has been completed revisited: its original Hodgkin-Huxley formulation has been replaced by a new Markov model (Figure 1), similar to the one used by Decker-Rudy for canine epicardial cells [10].

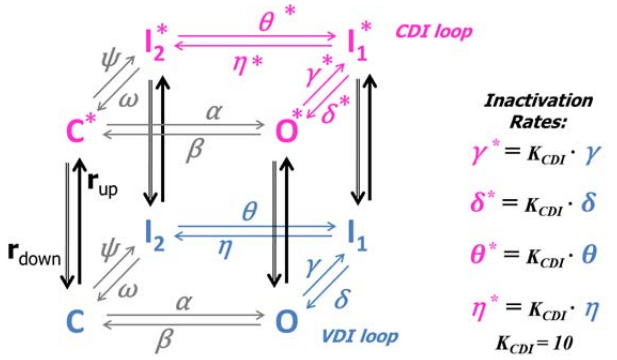

Figure 1. The new I_{CaL} Markov model: VDI and CDI are implemented in two V-dependent loops, connected by up/down rates. CDI is 10 times faster than VDI.

This Markov model consists of two structurally identical loops, each including 4 transitions: activation (from C to O), fast inactivation (from O to I_1), slow inactivation (from I_1 to I_2) and recovery (from I_2 to C).

Activation and recovery rates are exactly the same in the two loops; they have been directly derived from the ORd time constant and steady state values of the corresponding gating variables. As for fast and slow inactivation, rates on the CDI loop are 10 times faster than the ones in VDI loop.

All transition rates, even the ones on the CDI loop, are actually V-dependent [11]: Ca^{2+} concentration modulates the transitions between VDI/CDI loops only, by means of the n gate, used to calculate the r_{up}/r_{down} rates.

In the ORd model, the n gate represents the fraction of channels operating in CDI mode, and it is the only state variable, among the ones involved in I_{CaL} kinetics, which is directly dependent on intracellular $[Ca^{2+}]$. The n formulation is based on the interaction between Ca^{2+} and Calmodulin (CaM) bound to L-type Ca^{2+}-channels (Figure 2) and has been preserved in the modified model, even if kinetic rates have been slightly modified, in order to increase its sensibility to $[Ca^{2+}]$ variations.

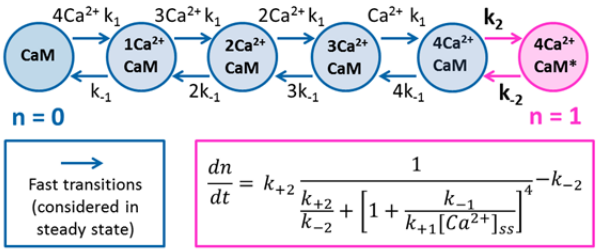

Figure 2. n gate kinetics (*modified from* [9]) and its corresponding differential equation.

In addition to I_{CaL} formulation, other minor changes were needed to refine the modified model, e.g. Ca^{2+} diffusion inside the sarcoplasmic reticulum has been speeded up, according to [12].

Model differential equations were implemented in Matlab (Mathworks Inc., Natick, MA, USA) and solved with a variable order solver (ode15s), based on numerical differentiation formulas [13]. Simulations were run with the original and modified models at variable $[Ca^{2+}]_o$ in the clinically relevant range 0.6-3 mM. Pacing at 1 Hz was maintained until steady state AP was reached (1000 s) and APD was measured as the interval between AP upstroke and the 90% repolarization level (APD_{90}).

3. Results

3.1. Model validation

The modified model has been validated against the same experimental data proposed for the original ORd model, especially the ones concerning I_{CaL} dynamics.

Different V-clamp protocol have been reproduced in simulations, and the modified model results were found in agreement with both the corresponding experimental data and the original ORd model.

I_{CaL} steady state activation, inactivation and I-V curves have been compared with data from Magyar et al. [14] (Figure 3A, 3B and 3C). Recovery from inactivation has been evaluated using the P_1P_2 protocol, as in Fulop et al. [15] (Figure 3D). Experimental results of CDI blocks were in agreement with the ones reported in the ORd paper [9], measured when considering Ba^{2+} current: in absence of CDI, I_{CaL} inactivation is much slower than in presence of both CDI and VDI (not shown).

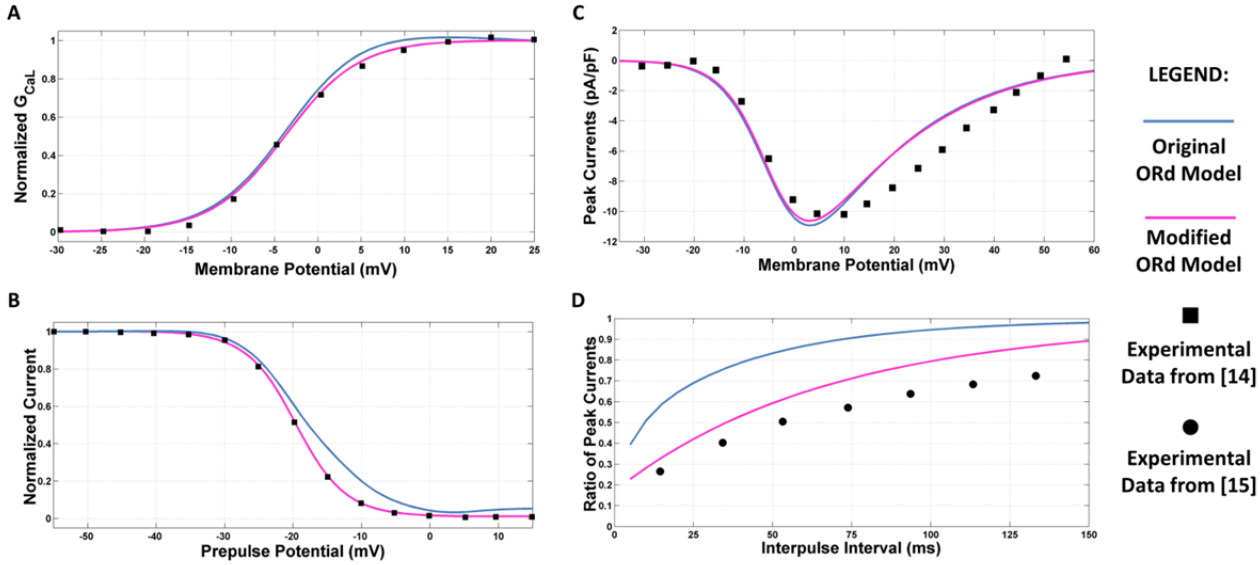

Figure 3. Simulated V-clamp protocols: comparison between the original and modified ORd model and experimental data [14,15]. **A)** I-V curve; **B)** Steady state activation; **C)** Steady state inactivation; **D)** Recovery from inactivation.

3.2 APD-$[Ca^{2+}]_o$ dependence

When $[Ca^{2+}]_o$ was set to the control value (1.8 mM), the modified and the original ORd models provided almost the same simulation results (Fig. 4A, solid line): ionic currents and AP were very similar in shape and length. In the modified model, I_{CaL} during AP plateau had a lower amplitude, due to the increased CDI.

When $[Ca^{2+}]_o$ variations have been simulated, however, significant differences were found in the two model results, both for AP and I_{CaL} (Fig. 4A, dashed and dotted lines). In the original ORd model, when $[Ca^{2+}]_o$ was set to higher values, the increase in driving force caused a larger I_{CaL}, which in turn lengthened AP. In the modified model, instead, the increase in driving force was compensated by the higher CDI, and the corresponding AP was shorter than in control. Therefore, only in the modified model the inverse relationship between APD and $[Ca^{2+}]_o$ was reproduced correctly (Fig. 4B).

3.3 Possible applications

The modified ORd model can be used in all clinical contexts where electrolyte variations occur, in order to assess a possible increased risk of arrhythmias for patients. A typical case of study is haemodialysis therapy, where patients regularly undergo relevant electrolyte changes (especially Ca^{2+} and K^+) in a few hours.

This kind of analysis had already been performed in a previous study [7], using experimental electrolyte data acquired during hemodialysis sessions as input of another human ventricular AP model [16].

Considering consistency between APD changes and QTc, simulation results had been compared with ECG data. The same comparison has been performed using the modified ORd model presented in this work, and simulation results are shown in Figure 5.

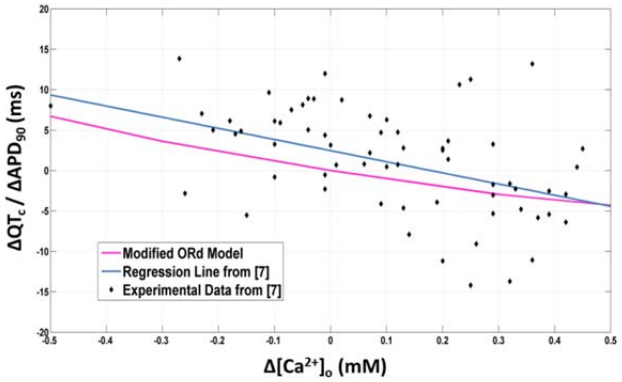

Figure 5. Analysis of the correlation between QT_c and simulated APs with different $[Ca^{2+}]_o$ levels (experimental data from [7], acquired during hemodialysis sessions). $[Ca^{2+}]_o$ variations are related to the mean experimental value (1.2 mM) and QT_c/APD_{90} are expressed as percentage variations to enable a direct comparison.

Another possible application is the head-down bed-test, a ground-based experiments which is used to induce and analyse microgravity effects on the cardiovascular system: during bed-rest blood electrolyte concentrations changes over time, with possible impact on cardiac repolarization [17].

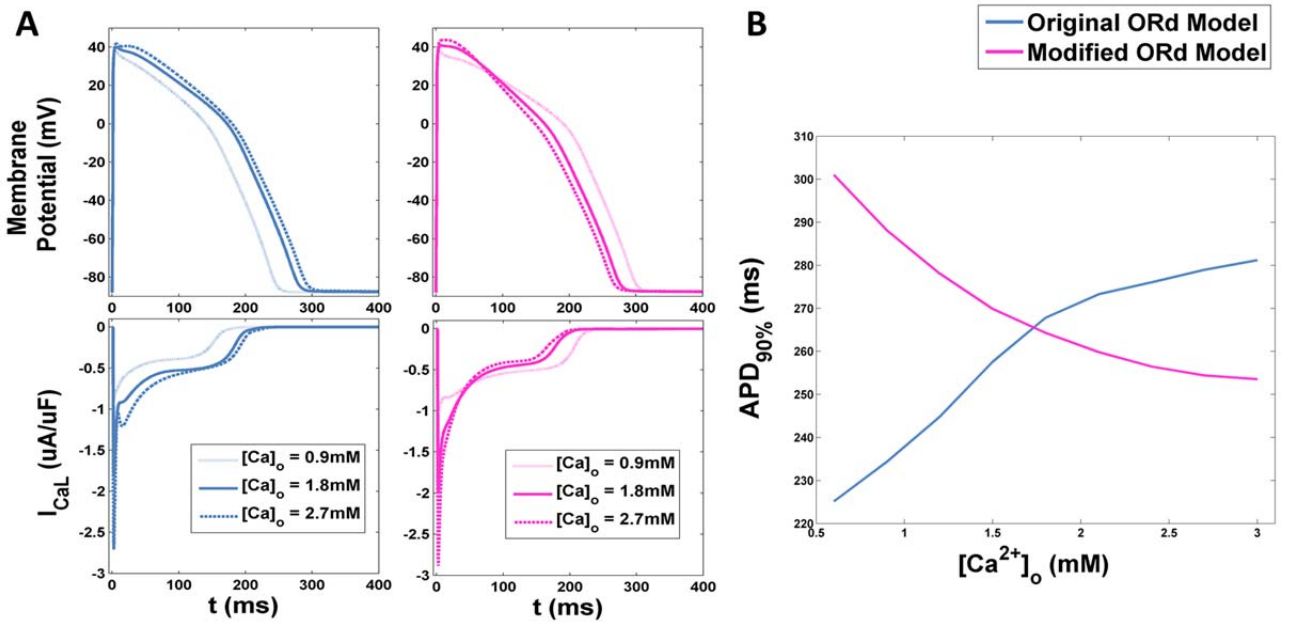

Figure 4. Comparison of the APD-$[Ca^{2+}]_o$ relationship for the original and the modified ORd models. **A)** Model APs and ICaLs, using three different $[Ca^{2+}]_o$; **B)** The APD-$[Ca^{2+}]_o$ relationship in the clinically relevant range 0.6-3.0mM.

4. Discussion and conclusions

APD dependency on extracellular Ca^{2+} concentration has been analysed in the most recently published human ventricular AP mathematical model [9]. Since this model does not reproduce properly the inverse APD-$[Ca^{2+}]_o$ dependence, observed both in vitro and in vivo [6], some modifications have been implemented, in order to improve its response to $[Ca^{2+}]_o$ changes.

L-type Ca^{2+} current has been replaced by a new Markov model, and CDI mechanisms has been strengthened with respect to VDI. Both inactivation processes have been implemented as V-dependent, the former 10 times faster than the latter: therefore, CDI in the modified model works simply as a faster VDI.

The modified model has been validated against the I_{CaL} experimental data used for the original one, in order to verify consistency between the two models in control conditions. Response to extracellular $[Ca^{2+}]_o$ in the 1-3mM range has been considered, and the modified model succeeded in reproduce the proper variations on APD.

Moreover, since modification involved mostly I_{CaL} CDI strengthening, this suggested that this inactivation mechanism may be underestimated in cardiac models.

The modified model may be used to explore a variety of contexts where electrolyte changes occur, e.g. haemodialysis sessions or Bed-Rest experiments, in order to assess the possible arrhythmic risk for patients.

5. Limitations and future developments

APD restitution (S_1S_2 V-clamp protocol) is not fully reproduced by the modified model presented here. This limitation seems related to the formulation of Ca^{2+} handling in the sarcoplasmic reticulum, which is heavily rate dependent. Therefore, slight modifications to Ca^{2+} release and uptake formulations are already in progress and hopefully this issue should be fixed in the near future.

References

[1] Hoffman BF, Suckling EE. Effect of several cations on transmembrane potentials of cardiac muscle. Am J Physiol 1956;186:317–24.

[2] Leitch S. Effect of raised extracellular calcium on characteristics of the guinea-pig ventricular action potential. Journal of Molecular and Cellular Cardiology 1996;28:541–51.

[3] Bai C-X. Role of nitric oxide in Ca2+ sensitivity of the slowly activating delayed rectifier K+ current in cardiac myocytes. Circulation Research 2004;96:64–72.

[4] Kass RS, Tsien RW. Control of action potential duration by calcium ions in cardiac Purkinje fibers. The Journal of General Physiology 1976;67:599–617.

[5] Temte J V., Davis LD. Effect of calcium concentration on the transmembrane potentials of Purkinje fibers. Circulation Research 1967;20:32–44.

[6] Severi S, Corsi C, Cerbai E. From in vivo plasma composition to in vitro cardiac electrophysiology and in silico virtual heart: the extracellular calcium enigma. Philosophical Transactions Series A, Mathematical, Physical, and Engineering Sciences 2009;367:2203–23.

[7] Severi S, Grandi E, Pes C, Badiali F, Grandi F, Santoro A. Calcium and potassium changes during haemodialysis alter ventricular repolarization duration: in vivo and in silico analysis. Nephrology, Dialysis, Transplantation 2008;23:1378–86.

[8] Grandi E, Pasqualini FS, Pes C, Corsi C, Zaza A, Severi S. Theoretical investigation of action potential duration dependence on extracellular Ca2+ in human cardiomyocytes. Journal of Molecular and Cellular Cardiology 2009;46:332–42.

[9] O'Hara T, Virág L, Varró A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. PLoS Computational Biology 2011;7:e1002061.

[10] Decker KF, Heijman J, Silva JR, Hund TJ, Rudy Y. Properties and ionic mechanisms of action potential adaptation, restitution, and accommodation in canine epicardium. American Journal of Physiology Heart and Circulatory Physiology 2009;296:H1017–26.

[11] Kim J, Ghosh S, Nunziato DA, Pitt GS. Identification of the components controlling inactivation of voltage-gated Ca2+ channels. Neuron 2004;41:745–54.

[12] Picht E, Zima A V, Shannon TR, Duncan AM, Blatter LA, Bers DM. Dynamic calcium movement inside cardiac sarcoplasmic reticulum during release. Circulation Research 2011;108:847–56.

[13] Shampine LF, Reichelt MW. The MATLAB ODE Suite. SIAM Journal on Scientific Computing 1997;18:1–22.

[14] Magyar J, Szentandrássy N, Bányász T, Fülöp L, Varró A, Nánási PP. Effects of thymol on calcium and potassium currents in canine and human ventricular cardiomyocytes. British Journal of Pharmacology 2002;136:330–8.

[15] Fülöp L, Bányász T, Magyar J, Szentandrássy N, Varró A, Nánási PP. Reopening of L-type calcium channels in human ventricular myocytes during applied epicardial action potentials. Acta Physiologica Scandinavica 2004;180:39–47.

[16] Ten Tusscher KHWJ, Noble D, Noble PJ, Panfilov A V. A model for human ventricular tissue. American Journal of Physiology Heart and Circulatory Physiology 2004;286:H1573–89.

[17] Passini E, Pellegrini A, Caiani EG, Severi S. Computational analysis of head-down bed rest effects on cardiac action potential duration. Computing in Cardiology 2013;40.

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