Computational Analysis of Extracellular Calcium Effects on an Improved Human Ventricular Action Potential Model

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A decrease in extracellular calcium concentration ($[Ca^{2+}]_o$) prolongs the action potential (AP) in ventricular cardiomyocytes, and vice versa. Although this phenomenon can be relevant to arrhythmogenesis in clinical settings, it is not included in most of the commonly used computational models of ventricular AP.

Therefore, the aim of this study has been to improve a recently published human ventricular model (O'Hara-Rudy, 2011), in order to reproduce the inverse relationship between $[Ca^{2+}]_o$ and AP duration (APD).

The original L-type Calcium current (I_{CaL}) formulation has been replaced by a minimal Markov chain, developed on purpose. Some minor modifications were also implemented to preserve the physiological behavior of the whole cell and to maintain consistency with the experimental data reported in the original paper.

Thus, the modified model can be used as a framework to explore the impact of $[Ca^{2+}]_o$ imbalances on the myocyte electrical activity. Further modifications are foreseen to improve model behavior in a larger variety of contexts.

1. Introduction

The effects of extracellular concentration of ionized calcium ($[Ca^{2+}]_o$) on cardiac electrophysiology have been already discussed in previous works [1-3]: as a general rule, elevated $[Ca^{2+}]_o$ levels shorten AP while low ones lengthen it, as observed in different species [4-8] and in human atrial cells [1]. Although data on the effects of hypo- and hyper-calcaemia on human ventricular APs are not available, the consistency between APD changes and corrected QT interval (QT_c) can be considered.

QT_c prolongation is associated with an increased risk of early after-depolarization and triggered arrhythmias, and the same applies to abnormal QT_c shortening: therefore Ca^{2+} dependency of repolarization is very important in all clinical contexts where electrolyte changes are involved, e.g. for hemodialysis patients.

From earlier studies, it is well known that the L-type Ca^{2+} current (I_{CaL}) is the ionic current mainly affected by $[Ca^{2+}]_o$ changes [2], even if there are many other ionic mechanisms involved and the phenomenon is still not completely understood: in fact, the change in driving force resulting from $[Ca^{2+}]_o$ increase, alone, should enhance calcium influx; nevertheless, calcium dependent inactivation (CDI) seems to play the major role, thus reducing APD.

A useful approach to investigate these complex interactions is the use of AP mathematical modeling, which allows to analyze channel alterations both individually and collectively: unfortunately, most of the published human ventricular AP models respond in an opposite way to $[Ca^{2+}]_o$ modifications, including the most recent ones [9-10].

The aim of this study has been the improvement of the O'Hara-Rudy (ORd) model [9] in order to properly reproduce the APD-$[Ca^{2+}]_o$ inverse relationship, as already done for older models in previous works [1-3].

2. Methods

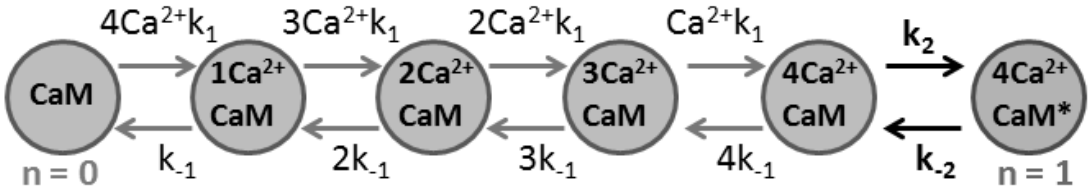
The ORd model of human ventricular myocyte [9] provided the basis for simulations in this study. Since ICaL is the current mainly affected by $[Ca^{2+}]_o$ changes, its formulation has been revisited, especially its modulation of calcium-dependent (CDI) and voltage-dependent (VDI) inactivation.

2.2. Ca^{2+} modulation: n gate

One of the features of ORd model, based on experiments by Kim et al. [11], is that CDI has been considered to function as a faster VDI, activated by high calcium concentration in the subspace region ($[Ca]_{ss}$).

Thus, both VDI and CDI mechanisms are voltage-dependent and a new state variable has been introduced to modulate them: n gate. It represents the fraction of channel operating in CDI mode, and it is the only state variable, among the ones involved in ICaL kinetics, to be directly dependent on $[Ca]_{ss}$. The n formulation, based on interaction between Ca^{2+} and Calmodulin bound to L-type Ca^{2+}-channels (Fig.1A), has been maintained, even if some of the kinetic rates have been modified in order to increase the sensibility to $[Ca]_{ss}$ variations (Fig. 1B).

A



B **n GATE DIFFERENTIAL EQUATION:**

$$\frac{dn}{dt} = k_{+2}\frac{1}{\frac{k_{-2}}{k_{+2}} + \left(1 + \frac{k_{-1}}{k_{+1}}\frac{1}{[Ca^{2+}]}\right)^4} - k_{-2}n$$

Original Model:		*Modified Model:*	
$\frac{k_{-1}}{k_{+1}} = 0.0002$	$jca_\infty = \frac{1}{1+e^{\frac{V+19.58}{3.696}}}$	$\frac{k_{-1}}{k_{+1}} = 0.0015$	$jca^*_\infty = \frac{1}{1+e^{\frac{V+69.58}{3.696}}}$
$k_{+2} = 1000$		$k_{+2} = 1000$	
$k_{-2} = jca$	$\frac{djca}{dt} = \frac{jca_\infty - jca}{\tau_{jca}}$	$k_{-2} = jca^*$	$\frac{djca^*}{dt} = \frac{jca^*_\infty - jca^*}{\tau_{jca^*}}$
$\tau_{jca} = 75$		$\tau_{jca^*} = 4$	

Figure 1. A) Schematic diagram for the n gate, representing the fraction of L-type Ca^{2+} channels undergoing CDI (adapted from [9]): Calmodulin (CaM), constitutively attached to L-type channels, interacts with Ca^{2+} ions with rate k_1; when four Ca^{2+} ions are bound, the complex can activate CDI mode ($4Ca^{2+}$/CaM*), with rate k_2. B) Differential equation for gate n [9]: the original transition rates have been slightly modified, in order to increase the sensibility to $[Ca^{2+}]$ changes in the subspace.

2.3. L-type Ca^{2+} current

The L-type Ca^{2+} current has been completely replaced by a minimal Markov chain with only 4 states, developed on purpose (Fig. 2A), instead of the originally implemented Hodgkin-Huxley formulation.

Inactivation process has been represented by two different inactivated states (I_1 and I_2): the transition between open state (O) and I_1 is faster than the one between I_1 and I_2, thus reproducing a bi-exponential dynamics. Transitions between I_2 and the closed state (C) and between C and O, represent recovery from inactivation and activation, respectively.

As a first step, transition rates have been set considering the original model time constants and steady state values of gating variables as basis (Fig. 2B); then, they have been slightly tuned in order to speed up inactivation process and particularly enhance CDI.

Ca^{2+} modulation acts directly on inactivation process, since both the transitions involved (O-I_1, I_1-I_2) are speeded up in CDI mode (Fig. 2C). It indirectly affects also recovery, since the rates of the I_2-C transitions were set by ensuring the microscopic reversibility [12].

Other minor modifications have been included in order to maintain the physiological behaviour of the whole cell. E.g. rapid delayed rectifier K^+ current was scaled to provide correct APD in control, as already done by the authors of the original ORd model.

Model differential equations were implemented in Matlab (Mathworks Inc., Matick, 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 1-3mM. Pacing at 1Hz was maintained until a steady state AP was reached and APD was measured as the interval between AP upstroke and the 90% repolarization level (APD_{90}).

Other simulation protocols have been implemented in order to verify the consistency of the modified model with the original one, e.g. I-V curve, recovery from inactivation, and analysis of I_{CaL} in presence of VDI only or both VDI and CDI.

3. Results

When $[Ca^{2+}]_o$ was set to the control value (1.8mM), the modified and the original ORd models presented almost the same simulation results (Fig. 3A, solid line). In particular, ionic currents and AP were very similar in shape and length. Only I_{CaL} had a lower amplitude in the modified model during phase 2, due to the increased CDI.

Changes in $[Ca^{2+}]_o$, however, produced two opposite effects, both on AP and I_{CaL} (Fig. 3A, dashed and dotted lines). In the original model, when Ca^{2+} was set to higher values, the increase in driving force caused a larger I_{CaL}, which in turn lengthened AP. In the modified model, the increase in driving force is compensated by the higher CDI, so that the final AP was shorter with respect to control. Therefore, only in the modified model the inverse relationship between APD and $[Ca^{2+}]_o$ is reproduced correctly (Fig. 3B).

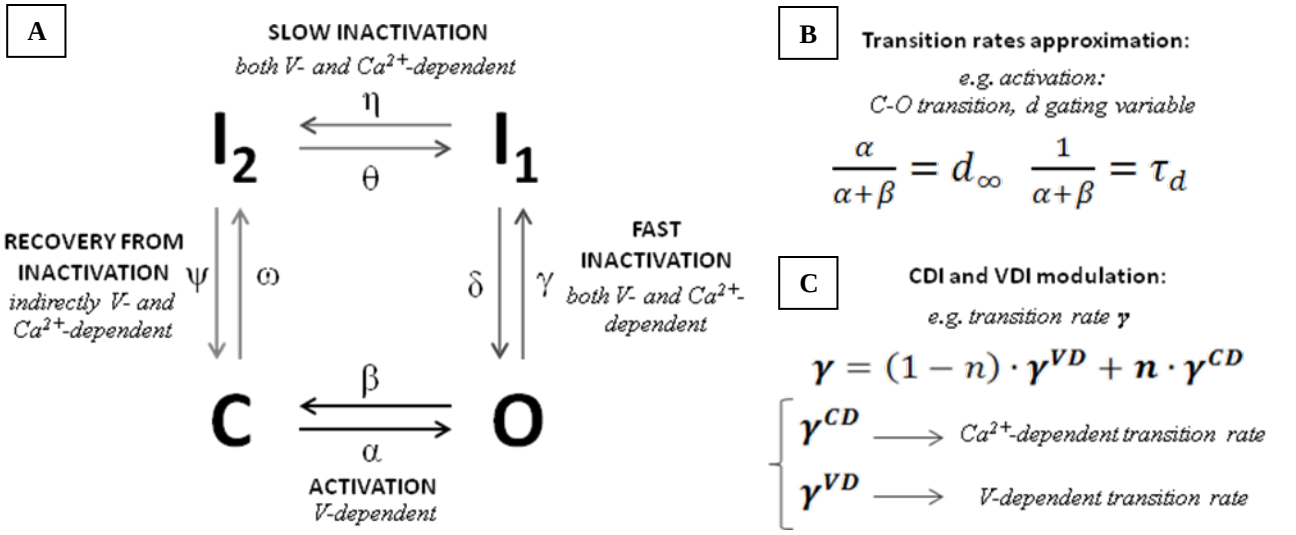


Figure 2. A) Markov chain used to reproduce I_{CaL} in the modified model. There are only 4 states: closed (C), open (O), fast inactivated (I_1) and slowly inactivated (I_2). Ca^{2+} acts directly on both inactivation processes, and it indirectly affects recovery, in order to ensuring microscopic reversibility. B) As a starting point, transition rates have been assigned from steady states and time constants of the original ORd model gating variables of I_{CaL}, using approximate equations. C) In order to include Ca^{2+} modulation, transition rates for inactivation processes have been set as a combination of two different rates, respectively VD and CD. CD-rate is faster than VD-rate, and their relative weights depend on n gate.

In order to validate the modified model, different voltage protocols have been considered.

Concerning the I-V curve, the simulation produced almost the same results for the two models (Fig.4A), even if the peak amplitude of I_{CaL} was slightly increased in the modified ORd model, with respect to the original one.

Considering the analysis of I_{CaL} modulation by Ca^{2+}, the same voltage protocol has been considered in two different conditions: control (CDI+VDI) and absence of CDI (VDI-only).

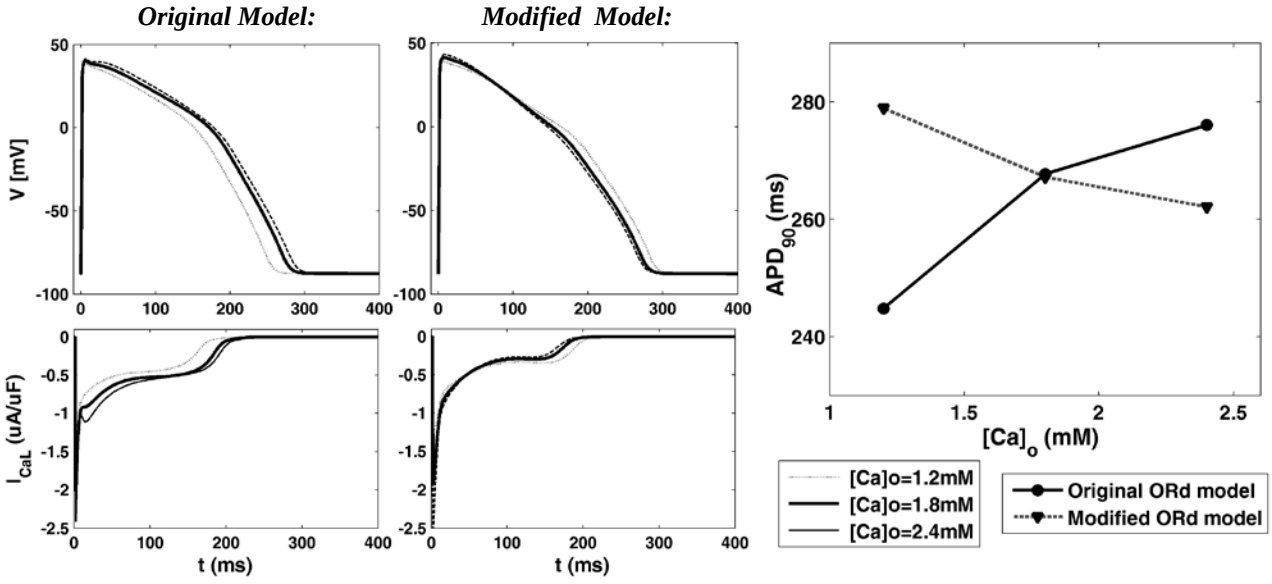


Figure 3 A) Simulation results for the original and the modified ORd models: AP and I_{CaL} are very similar in control conditions ($[Ca^{2+}]_o$=1.8mM) but they respond to $[Ca^{2+}]_o$ changes in an opposite way. B) APD changes as a function of $[Ca^{2+}]_o$ for the original and the modified ORd model: only the modified model is able to correctly reproduce the inverse relationship between the two considered variables.

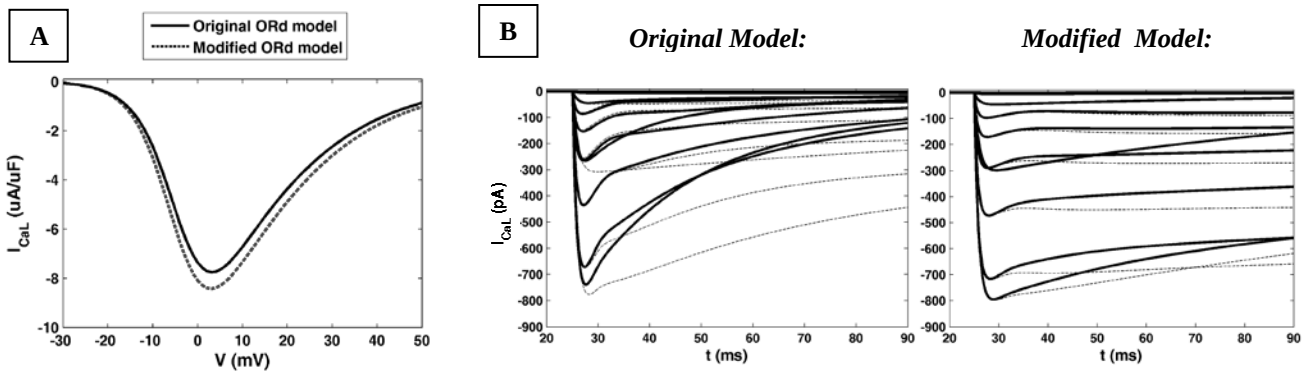


Figure 4 A) I-V curves for the original and the modified ORd model (solid and dashed line, respectively): the trend is very similar, even if ICaL peaks are a bit larger in the modified model. B) V-clamp ICaL in control conditions (CDI+VDI, solid lines) and in absence of CDI (VDI-only, dashed lines): in both models, the inactivation process is much slower in absence of CDI, even if in the modified model inactivation is always slower than in the original one.

The results were quite similar for the original and modified ORd model, in terms of Ca^{2+} effects: when CDI mechanisms was not present in the cell, I_{CaL} inactivation was much slower than in presence of both CDI and VDI. In the modified model, where CDI has been increased, this effect was more remarkable (Fig. 4B).

4. Conclusions

APD dependency on extracellular calcium concentration has been analyzed using a recently published human ventricular AP mathematical model, validated against a wide range of experimental data [9]. However, it did not reproduce correctly the inverse APD-$[Ca^{2+}]_o$ relationship, observed both *in vitro* and *in vivo*.

The original model has been modified, in order to solve this issue, and the modified model was actually able to respond to $[Ca^{2+}]_o$ changes in a proper way, without interfering with the behaviour of the whole myocyte.

Since the modifications involved mostly I_{CaL} CDI, as already verified with older AP models [1-3], this can be considered as the mechanisms more likely involved in real cells.

After a more comprehensive validation, the modified model could be used as a framework to explore effects on AP in all the different contexts in which electrolytes variations occur, e.g. haemodialysis patients or bed rest experiments.

References

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