In Silico Simulations of Experimental Protocols for Cardiac Modeling

Jesús Carro, José Félix Rodríguez, and Esther Pueyo

***Abstract*— A mathematical model of the AP involves the sum of different transmembrane ionic currents and the balance of intracellular ionic concentrations. To each ionic current corresponds an equation involving several effects. There are a number of model parameters that must be identified using specific experimental protocols in which the effects are considered as independent. However, when the model complexity grows, the interaction between effects becomes increasingly important. Therefore, model parameters identified considering the different effects as independent might be misleading. In this work, a novel methodology consisting in performing *in silico* simulations of the experimental protocol and then comparing experimental and simulated outcomes is proposed for parameter model identification and validation. The potential of the methodology is demonstrated by validating voltage-dependent L-type calcium current (I_{CaL}) inactivation in recently proposed human ventricular AP models with different formulations.**

Our results show large differences between I_{CaL} inactivation as calculated from the model equation and I_{CaL} inactivation from the *in silico* simulations due to the interaction between effects and/or to the experimental protocol. Our results suggest that, when proposing any new model formulation, consistency between such formulation and the corresponding experimental data that is aimed at being reproduced needs to be first verified considering all involved factors.

I. Introduction

From the earliest mathematical model of the action potential (AP) developed by Hodgkin and Huxley in the fifties, the complexity of current AP models has grown considerably. The advent of new experimental techniques has made an enormous set of experimental data readily available, which has motivated the development of more complex models to accurately describe cellular electrical activity. Whereas growing in model complexity is a natural consequence of the increased knowledge [1], the more complex the model, the more difficult the identification of model parameters tends to be.

A model of the AP involves the sum of different transmembrane ionic currents and the balance of intracellular ionic concentrations. Each ionic current follows a mathematical formula in which several effects are present, e.g., ion channel activation and inactivation gating. For each effect, a number of model parameters are identified based on data from experimental protocols specific of each particular ionic current. In most of the currently existing AP models in the literature, parameter identification is performed by assuming each effect as independent. At this point, validation of the identified parameters is partially done by comparing the prediction of the proposed mathematical model against the experiment. However, when the model complexity grows, the interaction between effects becomes increasingly important. Therefore, model parameters identified considering the different effects as independent might be misleading. To avoid this sort of problems new validation techniques that take into account combined effects within each ionic current formula are required.

In this work, a novel methodology for parameter model validation accounting for interaction effects is presented. The methodology consists in performing *in silico* simulations of the experimental protocol using the intended mathematical model of the effect and then comparing experimental and simulated outcomes. The proposed methodology can be used for validation and detection of inconsistencies between the tested model and the set of experimental data the model aims at representing, but also for model parameter identification. The potential of the methodology is here demonstrated by validating voltage-dependent L-type calcium current (I_{CaL}) inactivation in recently proposed human ventricular AP models. I_{CaL} inactivation plays a major role in the generation of arrhythmogenic afterdepolarizations in a number of disease states and conditions, and thus proper theorical characterization is crucial when investigating arrhythmia mechanisms.

Human ventricular cell models in the literature use different formulations for I_{CaL} inactivation. In the *ten Tusscher & Panfilov* (TP06) model [2], inactivation is modeled as the product of a fast f_2 and a slow f voltage-dependent gates, and a calcium-dependent gate f_{cass}. Gates have different time constants (τ_{f2}, τ_f and τ_{fcass}) and steady-state values ($f_{2\infty}$, f_∞ and $f_{cass\infty}$). In the *Grandi, Pasqualini & Bers* (GPB) model [3], voltage-dependent I_{CaL} inactivation is modeled by a single voltage-dependent gate f and two calcium-dependent gates, one for the subsarcolemmal compartment, $f_{Ca,sl}$, and another one for the junctional compartment, $f_{Ca,j}$. In the recently proposed *O'Hara & Rudy* (ORd) model [4] I_{CaL} inactivation is modeled as the multiplication and addition of voltage-dependent gates (f_f, f_{caf}, f_s, f_{Cas}, j) and a calcium-dependent gate n. The n gate is modeled as a Markov chain. All the other gates have different time constants, τ_{f_f}, τ_{f_s}, $\tau_{f_{caf}}$, $\tau_{f_{cas}}$, and τ_j, but the same steady-state value f_{ss}. All the above mentioned models use only one voltage-dependent I_{CaL} activation gate d, which multiplies the inactivation gating

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J. Carro is with School of Engineering, Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain: jcarro@usj.es

J. Carro, J.F. Rodríguez and E. Pueyo are with Aragon Institute for Engineering Research (I3A), Universidad de Zaragoza, Spain and with CIBER-BBN, Spain: {jfrodrig, epueyo}@unizar.es

J. Carro and E. Pueyo are with IIS Aragón, Spain.

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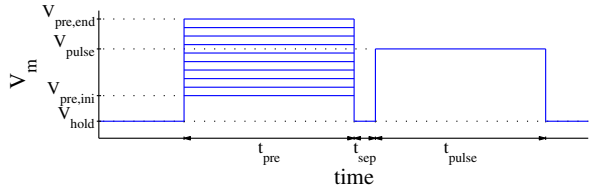


Fig. 1. Paired-pulse voltage clamp protocol.

TABLE I

PAIRED-PULSE TEST PARAMETERS IN EXPERIMENTAL PROTOCOLS

	Pelzman *et al* [5]	Li *et al* [6]	Magyar *et al* [7]
V_{hold} (mV)	-45	-80	-80
V_{pulse} (mV)	10	10	5
$V_{pre,ini}$ (mV)	-45	-100	-55
$V_{pre,fin}$ (mV)	40	60	15
ΔV_{pre} (mV)	5	10	5
t_{pre} (ms)	400	400	500
t_{sep} (ms)	10	5	0
t_{pulse} (ms)	400	300	390

expression in the I_{CaL} formulation.

II. METHODS

A. Paired-pulse protocol in experiments and simulations

Voltage-dependent I_{CaL} inactivation is experimentally characterized using the paired-pulse protocol, which is graphically illustrated in Fig. 1. This protocol consists in clamping the membrane potential from a holding potential V_{hold} to different pre-pulse potentials V_{pre} during a specified time interval t_{pre}. After this time, the potential is clamped to a pulse potential V_{pulse} during an interval t_{pulse}. Following this, the membrane potential is clamped back to V_{hold}. Variations of this protocol introduce a separation between the pre-pulse and the pulse potential, during which the potential is set to V_{hold} for a short period of time, t_{sep}, before clamping the membrane potential to V_{pulse}.

Different human ventricular cell models use different sets of experimental data to define their voltage-dependent I_{CaL} inactivation functions. The TP06 model uses data from [5], GPB from [6] and ORd from [7]. Table I shows the details of the experimental protocols used to characterize steady-state voltage-dependent I_{CaL} inactivation in human ventricular cardiomyocytes.

A MatLab program is implemented for each of the investigated AP models (Matlab R2008a, The MathWorks, Inc.). To simulate the experiments, intracelluar and extracelluar concentrations are fixed to the values used in the experimental procedures. All the currents except the I_{CaL} are blocked. By applying the inactivation protocol that has been used to identify the model parameters in each case, the *in silico* traces of the currents are obtained. This *in silico* protocol is performed using the same values of V_{hold}, V_{pre}, and V_{pulse} as in the corresponding experiments (see Table I). Also, free intracellular calcium is fixed to zero in the simulations to match the pipette solutions of the experiments. Calcium concentrations in all the other intracellular compartments follow the dynamics described in the corresponding model equations. Models are solved using the `ode15s` MatLab function with $dt = 0.1$ ms for the output.

B. Measurement of steady-state voltage-dependent I_{CaL} inactivation

Steady-state voltage-dependent I_{CaL} inactivation is obtained in the experiments and the *in silico* simulations during the pulse. In this part of the test, peak I_{CaL} current is measured for each potential used as pre-pulse and results are normalized by the value measured for the minimum pre-pulse potential. In this work we have also calculated the voltage-dependent steady-state inactivation as defined in each tested model.

C. Human ventricular cell models

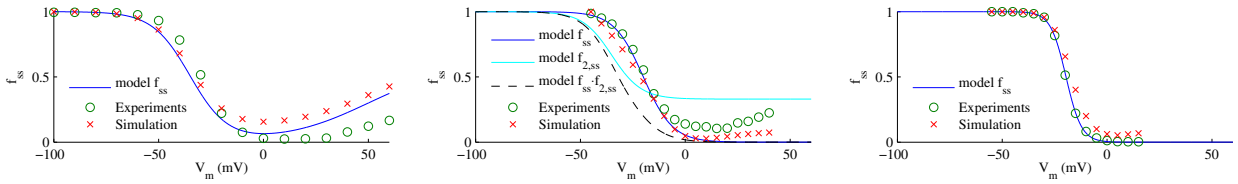
For the analysis of this work we have selected the GPB, TP06 and ORd models, each one representing I_{CaL} inactivation with a different formulation: i) with a single voltage-dependent gate (GPB model, $f \cdot d \cdot (A_j \cdot f_{Ca,j} + A_{sl} \cdot f_{Ca,sl})$); ii) as a multiplication of fast and slow voltage-dependent gates (TP06 model, $f \cdot f_2 \cdot d \cdot f_{cass}$); iii) as a weighted sum of fast and slow voltage-dependent gates (ORd model, $d \cdot ((A_{f_f} \cdot f_f + A_{f_s} \cdot f_s) \cdot (1-n) + j \cdot (A_{f_{caf}} \cdot f_{caf} + A_{f_{cas}} \cdot f_{cas}) \cdot n)$). In each case, A_x represents the coeficients used in the weighted sums.

III. RESULTS

Results of the paired-pulse protocol for steady-state voltage-dependent I_{CaL} inactivation are shown in Fig. 2. Each model is compared with the set of experimental data that was used to characterize voltage-dependent I_{CaL} inactivation.

Simulation results obtained with the GPB model fit quite well the model definition (see Fig. 2(a)). Differences are caused by the interaction with the voltage-dependent I_{CaL} activation gate d and by the protocol used to measured f_{ss}, as substantiated in the following. As shown in Fig. 3, the separation pulse makes a different effect depending on the pre-pulse potential. For $V_{pre} = -100$ mV, the effect is minimal because f_{ss} is very similar for V_{hold} and for this potential. On the other two cases shown, the value of f increases during the separation pulse because the value of f_{ss} for V_{hold} is larger than the values for $V_m = -20$ mV and $V_m = 60$ mV. For this reason, as shown in Fig. 2(a), differences between model definition and simulation increase when f_{ss} decreases. Interaction with voltage-dependent activation, represented by gate d, always reduces the measured value but the nomalization approximately corrects this behavior. Calcium-dependent inactivation gates have minimal effect, as these gates are almost completely open during most of the protocol duration. Finally, differences between experiments [6] and *in silico* simulations increase where differences between modeled and experimental data are larger.

The TP06 model is able to reproduce well enough the experiments from [5] (see Fig. 2(b)). In this case, the modeled steady-state voltage-dependent I_{CaL} inactivation is the product of the steady-states values ($f_{2,ss}$, f_{ss}) of the two inactivation gates (f_2, f). The results from the *in silico* simulations, which account for additional interaction effects, are closer to the experimental data due to: i) the effect of the



(a) (b) (c)

Fig. 2. Comparison between model definition (continuous and discontinuous lines), *in silico* simulations (red cross) and experimental results (green circles) for f_{ss}. a) GPB model. b) TP06 model. c) ORd model.

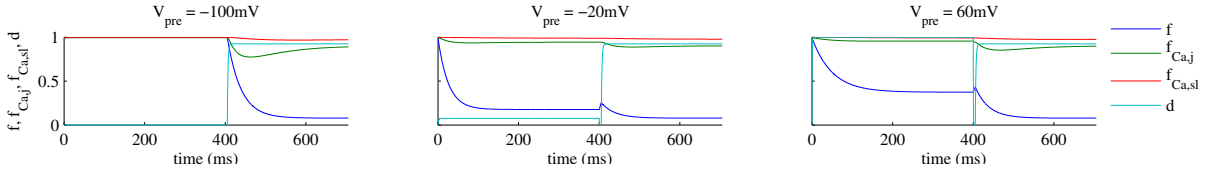


Fig. 3. Temporal evolution of the I_{CaL} gates (f, $f_{Ca,j}$, $f_{Ca,sl}$ and d) during the simulation of the GPB model.

separation pulse; ii) the fact that the slow I_{CaL} inactivation gate does not reach steady-state during the pre-pulse; iii) the fact that the minimum pre-pulse, used for the normalization of the peak I_{CaL} current, is not low enough for inactivation gates to attain a value of 1. Fig. 4 shows that for the TP06 model, as for the GPB model, the separation pulse has no effect on the inactivation gates for low potentials, but it increases their values for high potentials. In particular, the slow inactivation gate f does not reach steady-state for high potentials (see Fig. 4 for $V_{pre} = -5\ mV$ and for $V_{pre} = 40\ mV$). Fig. 4 also shows that calcium-dependent inactivation has not much effect, being this slightly more pronounced at lower potentials. Finally, one of the most visible differences between the model outcome (multiplication of the steady-state values of the gates) and the result of the *in silico* simulations is that the curves are shifted. This shift makes the *in silico* simulations approximate the experimental behavior due to the performed peak current normalization, which is made at a potential where the multiplication of the steady-state values is less than one.

Simulation results with the ORd model are off the experimental data in the center of the curve (see Fig. 2(c)). The largest difference appears when the pre-pulse potential is $-15\ mV$. The open probability at this potential is 18% larger than that obtained in the experiments. Evolution of the voltage-dependent I_{CaL} gate variables during the pre-pulse potential is shown in Fig. 5. This figure shows that for medium and low potentials, the n gate is practically not activated and the effects of the gates f_s and f_f dominate. On the contrary, for high potentials, the effects of the gates f_{caf}, f_{cas} and j prevail. This figure also shows that for medium and high potentials, the fast inactivation gates reach the steady-state value during the prepulse. However, the slow inactivation gates are not able to reach the steady-state value for the duration of the pre-pulse. For high pre-pulse potentials, the predominant gate f_{ca} is multiplied by j, which is able to reach steady-state. The effects of this product attenuate the discrepancies between simulations and experiments for high potentials, while in the medium of the curve the discrepancy is very pronounced.

IV. Discusion

A methodology amenable for validating computational model formulations has been introduced. The proposed methodology consists in performing *in silico* simulations using the same protocol as in the experiments that want to be reproduced, thus considering all variables involved in the model formulation as well as their interactions. The methodology has been applied to validate voltage-dependent inactivation of I_{CaL} in three human ventricular AP models presenting different formulations. Our results show large differences between I_{CaL} inactivation as calculated from the model equation and I_{CaL} inactivation from the *in silico* simulations. This is due to the interaction between voltage-dependent I_{CaL} inactivation gating and additional effects like: other gates in the model (i.e. I_{CaL} activation gate), the protocol definition, or the duration of the voltage pulses used to calculate inactivation properties. This suggests that, when proposing any new model formulation, consistency between such formulation and the corresponding experimental data that is aimed to be reproduced needs to be first verified considering all involved factors.

A. Inactivation modeled with a single gate

The GPB model and the associated *in silico* simulations provide similar results to the experiments used to fit the steady-state value of the voltage-dependent inactivation gate. Differences between *in silico* simulations and experimental results come in this case mainly from the model fitting. Our results suggest that a simple model like this one, with only one voltage-dependent inactivation gate, suffices to reproduce the experimental behavior of steady-state voltage-dependent I_{CaL} inactivation [6].

B. Inactivation modeled as the product of fast and slow inactivation gates

In the TP06 model the product of the steady-state values of the two voltage-dependent I_{CaL} inactivation gates is very far from the experimental behavior. However, the results of the

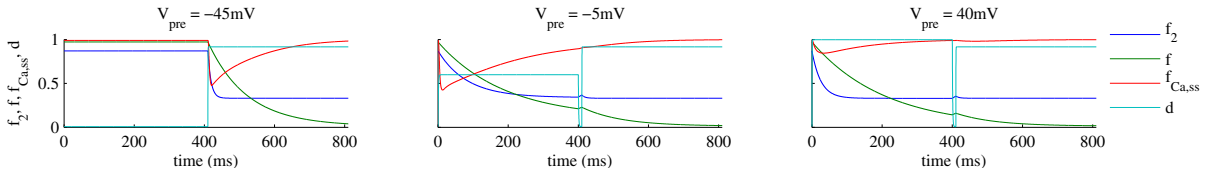


Fig. 4. Temporal evolution of the I_{CaL} gates (f, f_2, f_{cass} and d) during the simulation of the TP06 model.

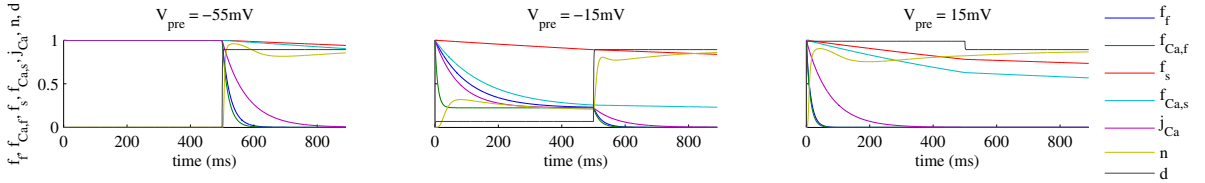


Fig. 5. Temporal evolution of the I_{CaL} gates (f_f, f_s, f_{caf}, f_{cas}, j, n and d) during the simulation of the ORd model.

in silico simulations with this model adequately reproduce the experimental observations. There are two effects that explain these results: i) the normalization by the peak I_{CaL} current at the minimum pre-pulse potential, as performed in [5], is done for a potential where the product of the two voltage-dependent inactivation gates is less than 1; ii) voltage-dependent inactivation gates do not reach the steady-state value at the end of the pre-pulse interval for some potentials.

C. Inactivation modeled as the weighted sum of fast and slow inactivation gates

In the ORd model, the definition of voltage-dependent I_{CaL} inactivation as a weighted sum of fast and slow inactivation gates is consistent with how experimentalists calculate inactivation time constants. As described in [4] and [5], time constants are calculated by fitting a biexponential function to the inactivation phase of the experimental current traces. Whereas this is true, large discrepancies between the ORd model simulations and the experiments of [7] used for model development are found for medium pre-pulse potentials.

There are two possible sources behind such discrepancies, related to: i) the time constant of the voltage-dependent slow inactivation gate; ii) the definition of voltage-dependent I_{CaL} inactivation as the sum of two gates.

The first source has to do with the fact that model time constants are identified in [4] based on a simple-pulse test protocol of 75 *ms* duration, whereas the slow time constant of voltage-dependent I_{CaL} inactivation in the ORd model is of the order of 10 seconds.

The second source has to do with formulating I_{CaL} inactivation as a sum of two gates. If inactivation is expressed as a product, when one of the gates reaches zero, the product reaches zero. Due to the interaction with the n and j gates in this model, as we have shown previously, the result for high potentials is the product of two gates and the effect is reduced, while this does not happen for low and medium potentials.

V. Conclusions

When developing AP models, parameter identification is typically performed by fitting the model equations to experimental data without ensuring consistency between the model definition and the hypothesis underlying the processed experimental data. As we have shown in this paper, proper characterization and validation of a given model should be performed with the *in silico* simulation of the experimental protocol. Proceeding this way, interaction effects between different model components are accounted for and model inconsistencies are avoided.

As a final remark, complex models represent a real challenge for parameter identification and validation. This does not mean that models should be necessarily simple, but that complex models require additional testing in order to fully verify their correct performance.

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