

Development, Implementation and Testing of a Multicellular Dynamic Action Potential Clamp Simulator for Drug Cardiac Safety Assessment

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Abstract

As drugs can be multichannel blockers it is important to assess their cardiac safety taking into account multiple currents. In silico action potential (AP) models have been proposed for being able to integrate drugs effect on ionic currents and generate the resulting AP. However, a mathematical description of drug effects is required, which could be inaccurate.

Dynamic Clamp has been proposed for drug cardiac safety assessment. In the dynamic action potential clamp (dAPC) configuration it creates a hybrid model connecting a real cell with a computer simulation. This way, drugs could be administrated directly to real cells, and effects on currents can be taken into account when generating the AP. Here we design and simulate a parallel multichannel dAPC system. The system includes the real cells overexpressing the currents of interest, the voltage clamp acquisition system, and the AP in silico model.

1. Introduction

Drug cardiac safety assessment is a fundamental step in drug development. In particular, it is essential to detect, in pre-clinical experiments, those drugs which could cause arrhythmias. As drugs can be multi-channels blockers, it is useful to assess their effect on multiple currents, but, given the complex interaction between currents during the action potential (AP), it can be difficult to understand the overall effect on the AP. In silico simulations can be useful, but a mathematical description of a drug's effect on each ionic current is needed, which may be incorrect or not exhaustive. The dynamic action potential clamp (dAPC) technique [1] could offer a solution as it creates a closed

circuit in real-time between the in silico model and in-vitro cells. Currents of interest could be acquired from the cells and given in input to the AP model, which computes the corresponding membrane potential (V_m) value. In this configuration, no drug description is required, as drugs can be administrated directly to the cells and their effects will reflect accordingly in the AP computed by the model. Moreover, as automated patch clamp systems are available, Dynamic clamp experiments can be performed with a high throughput rate [2].

In this project, we recreated a simulator of a multicellular dAPC system, incorporating each system component and the protocols needed, which could serve as a reference for predicting outcomes of future experiments. Moreover, given the presence of some undesirable effects from the acquisition system, it was possible to evaluate how such components and their compensations affect the AP computed by the in silico model. Lastly, we evaluated if it is possible to correctly quantify drug effects in dAPC. We determined those effects by calculating APD_{90} changes upon drug administration to the cells. Those results were then compared with the one obtained from virtually administering the same drugs to a ventricular cardiomyocyte simulated with the the O'Hara-Rudy (ORD) model [3].

2. Methods

The main system components to be modelled are: i) the real cells overexpressing the currents of interest, ii) the voltage clamp acquisition system, and iii) the action potential (AP) computational model to be numerically integrated in real time by the dAPC system.

2.1. Computational modelling of currents overexpressed in heterologous cells

We modelled three cells, each one overexpressing a different current: I_{CaL} , I_{Kr} and I_{K1} . In particular, we assumed cells to be small chinese hamster ovary (CHO) cells where each current was modeled with its ORd formulation [3] with a typical maximum conductance value for CHO cells. The K^+ reversal potential was the same for all cells and equal to the diastolic value in ORd. White noise was added to every current. In cells expressing I_{CaL} , intracellular Ca^{++} concentration was set equal to the diastolic value in ORd as it is constant given the presence of the pipette and the lack of sarcoplasmic reticulum in non-cardiomyocytes.

2.2. Computational modelling of the Voltage Clamp system

The acquisition system was modelled as in Lei *et al.* [4]. The system represents a whole-cell patch-clamp voltage-clamp amplifier setup with adequate compensations. Compensations, as written in the ODE system, include a capacitance compensation and a finite series resistance compensation.

2.3. AP computational model

The AP model we used is the ORd model [3]. Some model modifications were needed to perform a dAPC simulation. Firstly, I_{Kr} and I_{K1} equations were deleted as well as the activation gate equation for I_{CaL} and its permeability (P_{Ca}) value. On the other hand, equations for the driving force (Ψ_{Ca}), inactivation gates, and phosphorylation have been left. Then, $[K^+]_i$ is kept equal to its initial condition, consistently with $[K^+]_i$ in the CHO cells where it is supposed constant given the presence of the pipette.

Model differential equations were implemented in Simulink (Mathworks Inc., Natick, MA, USA) and solved with a variable order solver (ode15s), based on numerical differentiation formulas.

2.4. Implemented protocols

As the experiment is to be run in real-time, offline leakage current subtraction is not an option, so an online leakage compensation protocol needs to be included. Considering the leakage current as a linear function of the pipette potential (V_p) it is possible to estimate the two parameters needed (G_{leak} and E_{leak}) with a linear fitting applying a ramp voltage-clamp protocol, in a range of membrane potentials in which the current of interest is zero, so that the measured current must be a leakage current. Then, during the experiment, the estimated leakage current is subtracted from the measured current in real-time.

As currents are acquired from heterologous systems overexpressing the channel of interest, it is reasonable to expect a greater current amplitude than the one from the in silico model. Therefore, every current must be scaled before giving it in input to the AP model. The major issue with this step is that while we perfectly know the maximum conductance of the current of interest in ORd model, the maximum conductance of the in-vitro cell is unknown. The method we used to overcome this problem was to maximize the similarity between currents, the one from the in-vitro cell, and the one expected from ORd model, which was previously saved. Current from the cell is acquired in AP clamp with an AP saved from the ORd model, then the scaling factor (G_{scale}) is found by minimizing the mean square error between currents.

A different protocol has to be considered for I_{CaL} . The lack of calcium transient into the CHO cells causes, in turn, lack of calcium-dependent inactivation (CDI). Therefore, it wouldn't be accurate to include I_{CaL} in the AP model without CDI and with a driving force substantially different from the one in cardiomyocytes. To overcome this problem, we acquired I_{CaL} from the simulated CHO cell and divided the current for the estimated values of the Ψ_{Ca} and the inactivation gates. This way only $I_{CaL} = d \cdot P_{Ca}$ is given in input to the AP model. Then, in the ORd model, the rest of the equation is computed according to the $[Ca^{++}]_{ss}$ computed value:

$$I_{CaL} = G_{scale} \cdot \frac{I_{CaL,CHO}}{\Psi_{Ca,CHO} \cdot (f \cdot (1 - n) + f_{Ca} \cdot n \cdot j_{Ca})_{CHO} \cdot \Psi_{Ca,ORd} \cdot (f \cdot (1 - n) + f_{Ca} \cdot n \cdot j_{Ca})_{ORd}}$$

Therefore, not only the scaling factor has to be inferred, but also the $[Ca^{++}]_{ss,CHO}$ estimation, needed for CDI compensation, is required. This time, I_{CaL} is acquired from the cell with an AP clamp and serves as a reference. Then, both the scaling factor and $[Ca^{++}]_{ss}$ are estimated as the values which maximize the similarity between the measured current and the current from the AP model (calculated in AP clamp with a fixed value for $[Ca^{++}]_{ss}$) minimizing the mean square difference between them.

3. Results

3.1. Simulaton of multichannel Dynamic Clamp with perfect compensations

We performed a dAPC simulation with three different cells, each one expressing a different current, in a perfectly compensated acquisition system. Results are shown in Figure 1. The APD_{90} obtained in dAPC is 268.3 ms, to be compared to 270.6 ms as in the original ORd model.

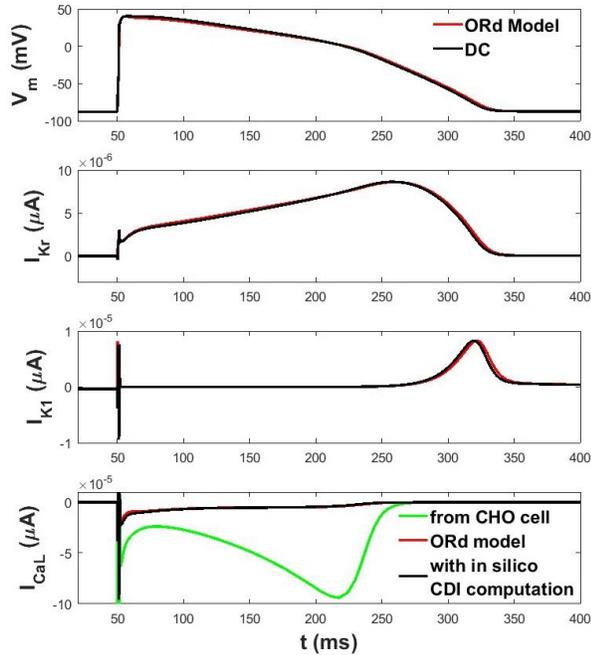


Figure 1. AP computed in dAPC and currents acquired from cells multiplied by their own scaling factor (in black), compared with AP and currents from ORd (in red).

3.2. Sensitivity to incomplete compensations

In the acquisition system, there are many undesirable effects worsening the quality of the measurement, which are therefore compensated. These compensations will never be perfect, so in the simulation, we observed how a variation in compensations affects the APD_{90} in dAPC.

Membrane capacitance: the presence of the membrane capacitance and its incomplete compensation can affect the scaling factor estimation protocol and the APD_{90} as seen in Figure 2, black line.

Series resistance: the presence of finite series resistance and its compensation has only milder effects as seen in Figure 2, blue line.

Voltage Offset: a voltage offset, which remains after the bridge balance compensation performed before every experiment is run, has a significant impact on the APD_{90} in the simulation (Figure 3). Besides the need to do an optimal compensation before the experiment starts, this result suggests that the scaling factor estimation protocol should be performed considering only currents acquired during the systolic phase of the AP, as their diastolic value is more sensitive to the presence of an offset voltage.

Leak current: although the seal between the micropipette and the cell membrane must exhibit a resistance value higher than $1 \text{ G}\Omega$ to meet the quality standard, leak-

age compensation is still required. This compensation has a mild effect if R_{seal} has a high value, but it becomes essential for lower values as seen in Figure 4.

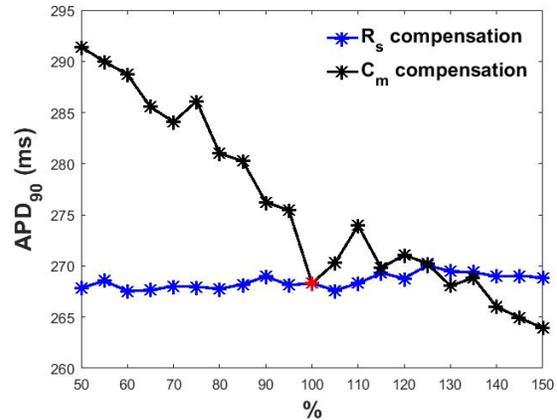


Figure 2. APD_{90} obtained at different percentage levels of C_m (in black) and R_s (in blue) compensation. In red, APD_{90} when C_m and R_s are perfectly compensated.

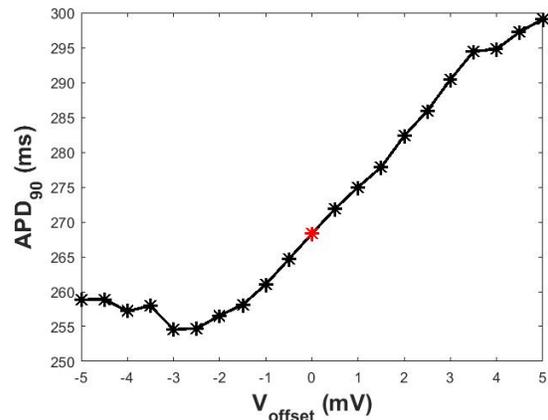


Figure 3. APD_{90} obtained in presence of different levels of V_{offset} . In red, APD_{90} when V_{offset} is zero.

3.3. Virtual drug administration

Drug administration was simulated by decreasing each maximum conductance by the corresponding percentage block (e.g. Verapamil: 6.75% for I_{Kr} , 47.76% for I_{CaL} , 0.96% for I_{K1} , at $EFTCP_{max} = 0.088 \mu\text{M}$) [5]. The effect was then assessed in terms of APD_{90} change after drug administration. Virtual administration of Verapamil in the perfectly compensated dAPC system induced $\Delta APD_{90} = -19.5 \text{ ms}$, compared to $\Delta APD_{90} = -14.8 \text{ ms}$ in the ORd model. Moreover, to evaluate if it is possible to assess drug

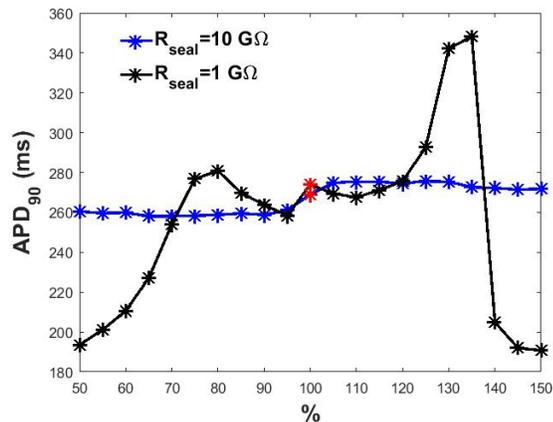


Figure 4. APD_{90} obtained at different percentage levels of I_{leak} compensation. In red, APD_{90} when I_{leak} is perfectly compensated.

effects in an experiment where variability between cells and wells occurs, we simulated 10 different experiments where cells and acquisition system parameters varied according to Table 1. In these experiments, $\Delta APD_{90} = -12.9 \pm 8.1$ ms.

Table 1. Parameters variation

Parameter	mean \pm standard deviation
G_{max}	$G_{max} \pm 20\%$
V_{offset}	2 ± 1 mV
R_s	$5 \pm 0,5$ MΩ
C_m	$10 \pm 0,5$ pF
R_{seal}	8 ± 4 GΩ

4. Discussion and Conclusions

We have successfully simulated a multicellular dAPC system. When the acquisition system was supposed to be ideal and all the artifact sources perfectly compensated, the APD_{90} computed dynamically by integrating the results from 3 cells overexpressing 3 different ionic currents was substantially the same of an isolated cell (simulated by the ORd model). The residual small difference (about 2 ms) is likely due to the unavoidable delay introduced in the digital feedback loop.

Importantly, a novel approach to manage the dynamic clamping of I_{CaL} , in which only the relevant (voltage-dependent) information acquired from the cell, lacking proper calcium handling, is integrated into the in silico AP model, in which the calcium-dependent features of the current are simulated, has been designed and implemented.

The main undesired effects that can be anticipated in the real system have been analysed and their effects have been

quantified, pointing out that the membrane capacitance, offset voltage and leakage current are the main responsible for changes in APD_{90} . The model dependency of the presented results should be tested by using different models both for the currents in the "real" cells, and for the "in silico" AP.

The simulation results fully support the feasibility of such an hybrid system for cardiac safety assessment. The developed simulator might be a useful tool for the design and implementation of the real system by predicting outcomes of future experiments.

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