Automaticity in Cardiomyocytes Derived From Human Induced Pluripotent Stem Cells as Result of Different Mechanisms

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Abstract

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are nowadays one of the most studied cell types, especially for their role as in vitro models to test drug safety. Here we propose an update of our recent Paci2018 hiPSC-CM model, focusing particularly on the mechanisms underlying the action potential (AP) automaticity, i.e. one of the clearest markers of hiPSC-CM immaturity. We used two recently published formulations of the fast Na^+ (I_{Na}) and the funny (I_f) currents to improve our previous fitting of in vitro experiments. We then used an optimization framework to automatically identify the parameters formalizing currents/mechanisms for which in vitro data were not available. We identified a model producing APs and Ca^{2+} transients (CaTrs) in agreement with literature and our in-house CaTr data. We showed that a strong (90%) Na^+/Ca^{2+} exchanger (I_{NCX}) block suppresses the spontaneous APs, in agreement with in vitro data. The new Paci2019 was further validated against the in vitro experiments used for the Paci2018 model, showing that this update did not affect the capability of the new model to simulate mechanisms (e.g. responses to drugs or proarrhythmic events) successfully reproduced by the previous model. In conclusion, the Paci2019 model represents an improved tools for in silico studies e.g. on hiPSC-CM responses to drugs.

1. Introduction

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are nowadays extensively studied, especially for their role as *in vitro* models to test drug safety. In the last decade, the amount of available *in vitro* data increased considerably, together with our understanding of hiPSC-CM electrophysiology. Experiments supported hiPSC-CM use as drug pre-screening models (e.g. in the *Comprehensive in Vitro Proarrhythmia Assay* initiative - CiPA), but also partly dampened the initial enthusiasm because of hiPSC-CM structural immaturity. Together with the increasing amount of *in vitro* data, more and more *in silico* studies helped to shed light on hiPSC-CM electrophysiology and responses to drugs [1–6].

Two mechanisms are currently considered responsible for automaticity in hiPSC-CMs: i) membrane potential depolarized by the funny current (I_f) [7] or ii) by the Na^+/Ca^{2+} exchanger (I_{NCX}) as consequence of Ca^{2+} release from sarcoplasmic reticulum (SR) [8]. Currently, there is no consensus, also because of contradicting *in vitro* experiments.

We observed a limitation in our recent Paci2018 hiPSC-CM model [3], i.e. a too strong window current for the fast Na^+ current (I_{Na}), which makes I_{Na} not only fundamental for the upstroke phase of the AP, but also in sustaining the automaticity of the spontaneous electrical activity. Because of this, the Paci2018 model failed to simulate the cessation of the spontaneous electrical activity in case of strong block of Na^+/Ca^{2+} exchanger (I_{NCX}) as shown by recent *in vitro* and *in silico* experiments [4,8].

Here we propose an update of our Paci2018 hiPSC-CM model [3], focusing particularly on the mechanisms underlying the action potential (AP) automaticity, i.e. one of the clearest markers of hiPSC-CM immaturity.

2. Methods

In order to improve the simulation of the spontaneous hiPSC-CM electrical activity we proceeded in three steps:

- replacement of I_{Na} and funny current (I_f) formulation and other minor changes;
- automatic parameter optimization;
- validation of the model against the *in vitro* experiments used to validate the Paci2018 model.

2.1. New ion current formulations

We first replaced the formulation of I_{Na} and of I_f, with new ones based on [4], that provided an improved fitting of the *in vitro* voltage-clamp experiments from [9,10].

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Since these changes suppressed the model automaticity, we manually tuned: the maximum Na^+/Ca^{2+} exchanger ($I_{NCX,max}$), the Na^+/K^+ pump ($I_{NaK,max}$), the maximum Na^+ and Ca^{2+} background current conductances ($G_{b,Na}$ and $G_{b,Ca}$) and the maximum SERCA uptake ($V_{max,up}$). This led to a model showing spontaneous electrical activity and biomarkers similar to those reported in [3].

2.2. Automatic parameter optimization

Since this intermediate model did not simulate all the AP and CaTr biomarkers simulated by the Paci2018 model within their *in vitro* ranges, we then run the same automatic optimization algorithm we used in [3]. The target AP *in vitro* biomarkers, taken from [9] and considered for the cost function, were: AP amplitude (APA), maximum diastolic potential (MDP), cycle length (CL), maximum upstroke velocity (dV/dt_{max}), AP duration at 10, 30 and 90% of repolarization (APD_{10}, APD_{30}, APD_{90}) and AP shape factor (Tri), i.e. the ratio $(APD_{30} - APD_{40})/(APD_{70} - APD_{80})$. The target Ca^{2+} transients (CaTrs) *in vitro* biomarkers, used for the Paci2018 model and recorded at BioMediTech (Tampere, Finland) [3], were: CaTr duration (CTD), time to peak ($tRise_{10,peak}$), rise time from 10% to 50% ($tRise_{10,50}$), rise time from 10% to 90% ($tRise_{10,90}$), decay time from 90% to 10% ($tDecay_{90,10}$) and the CaTr cycle length (CaTr CL). We optimized the same parameters as in [3]. The parameter values were constrained in a range [-20%, +20%] with respect to their nominal value in the Paci2018 model, in order to avoid non-physiological values.

2.3. Model validation

To validate the new hiPSC-CM model we considered the following data:

- administration of SEA0400 (I_{NCX} blocker) and ivabradine (I_f blocker) as in [8];
- spontaneous Ca^{2+} release from SR in case of Ca^{2+} overload [8] and due to abnormal Ca^{2+} handling;
- administration of tetrodotoxine (I_{Na} blocker), nifedipine (I_{CaL} blocker), E4031 (I_{Kr} blocker) and 3R4S-Chromanol 293B (I_{Ks} blocker) [9];

3. Results

3.1. Automaticity in the Paci2019 model

The replacement of I_{Na} and I_f and the parameter optimization produced the APs and CaTrs shown in Fig.1, whose biomarkers are reported in Table 1. Furthermore, in spite our CaTr biomarkers did not include absolute measurements of CaTr amplitude, in [11] they observed CaTr amplitude ranging from 30 to 160nM in hiPSC-CM ensembles incubated at 37°C (calibrated Fura-2-based photometry measures). Our model produces CaTrs with amplitude 160nM.

The main difference in terms of ion currents between the Paci2018 and the Paci2019 models is the I_{NCX} shape (Fig. 1). In fact, by reducing the I_{Na} window current and using a more accurate fitting of I_f, we unveiled a remarkable inward I_{NCX} inward component right before the AP upstroke, which significantly contributes to the membrane potential depolarization. This is indeed caused by the Ca^{2+} release from the sarcoplasmic reticulum (SR), which now assumes an important role in the model spontaneous electrical activity. Indeed, increasing blocks of I_{NCX} first slow down the rate of the spontaneous APs, until their suppression (Fig. 2), as observed *in vitro* as consequence of the I_{NCX} inhibitor SEA0400 [8]. As in [7,8], we tested also the effect of I_f block. The effect of a 41% block, corresponding to 3 μM ivabradine [4], induced only a slight reduction of AP rate (-7.5%) in agreement with [8]. However, for increasing blocks, the effect on AP rate was more relevant: 60% I_f block caused -12% AP rate and 80% block induced the cessation of the spontaneous APs after 260s from drug administration, in line with [7].

Table 1. AP and CaTr biomarkers: *in vitro* intervals used for parameter optimization and *in silico* values.

Biomarker	*In vitro* value (mean±SD)	*In silico* value
APA (mV) [9]	104 ± 6.0	102.0
MDP (mV) [9]	-75.6 ± 6.6	-74.9
AP CL (ms) [9]	1700.0 ± 547.7	1712.4
dV/dt_{max} (V/s) [9]	27.8 ± 26.3	20.4
APD_{10} (ms) [9]	74.1 ± 26.3	87.0
APD_{30} (ms) [9]	180 ± 58.6	223.8
APD_{90} (ms) [9]	414.7 ± 119.4	390.2
AP Tri (-)[9]	2.5 ± 1.1	2.8
CTD (ms) [3]	804.5 ± 188.0	691.5
CaTr $tRise_{10,50}$ (ms) [3]	82.9 ± 50.5	55.0
CaTr $tRise_{10,90}$ (ms) [3]	167.3 ± 69.8	118.2
CaTr $tRise_{10,peak}$ (ms) [3]	270.4 ± 108.3	184.0
CaTr $tDecay_{90,10}$ (ms) [3]	409.8 ± 100.1	341.0
CaTr CL (ms) [3]	1653.9 ± 630	1712.4

3.2. Delayed After-depolarizations

As for the Paci2018 model, we tested the Paci2019 capability of producing delayed after-depolarizations (DADs) in different conditions. We induced Ca^{2+} overload, by increasing the extracellular Ca^{2+} concentration (2.9 from 1.8mM) [12]. Fig. 3 shows how Ca^{2+} overload triggers spontaneous releases from SR that starts DADs (grey arrows) or even single and multiple anticipated APs. A similar result was obtained with a normal extracellular

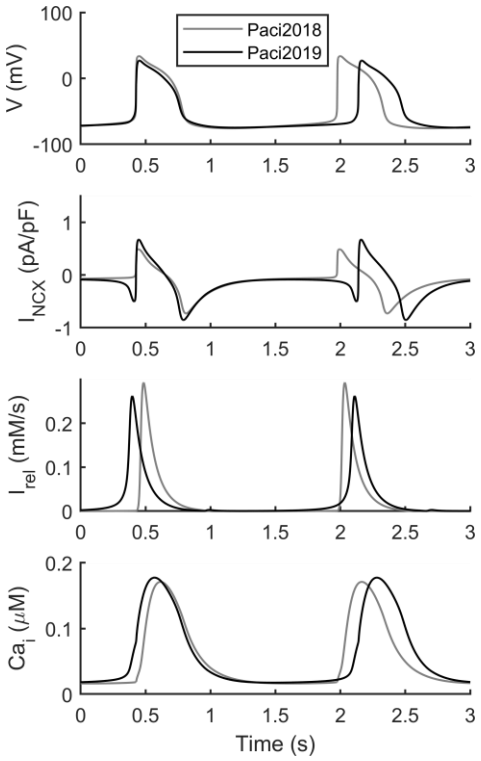

Figure 1. Paci2019 model. From top to bottom: APs, I_{NCX}, I_{rel} and CaTrs.

Ca^{2+} concentration (1.8mM) but simulating an abnormal RyR machinery (namely, by shifting $RyR_{o,half}$ and $RyR_{c,half}$ by −0.002 and 0.002mM respectively, doubling RyR_o time constant and reducing to half of its nominal value RyR_c time constant) (Fig. 4).

3.3. Response to current blockers

We tested the Paci2019 model with four current blockers tested *in vitro* in [9]: tetrodotoxin, E4031, nifedipine and 3R4S-Chromanol 293B. As the *in vitro* hiPSC-CM, the model was stimulated at a constant pacing rate 1Hz with depolarizing pulses of 5ms duration. In agreement with the *in vitro* experiments (i) tetrodotoxine delays the upstroke, (ii) E4031 prolongs APD, (iii) nifedipine shortens APD and (iv) 3R4S-Chromanol 293B has only minor effects on the AP shape (Fig. 5).

4. Conclusions

Automaticity is one of the main hiPSC-CM features and there is no consensus on its triggering mechanism (I_f vs I_{NCX}+Ca^{2+} releases). A reasonable hypothesis is that both mechanisms could coexist and the *in vitro* data supporting one or the other could be explained by the phenotypical variability (together with different *in vitro* setups, e.g. single cell data vs cell ensemble recordings). In this work

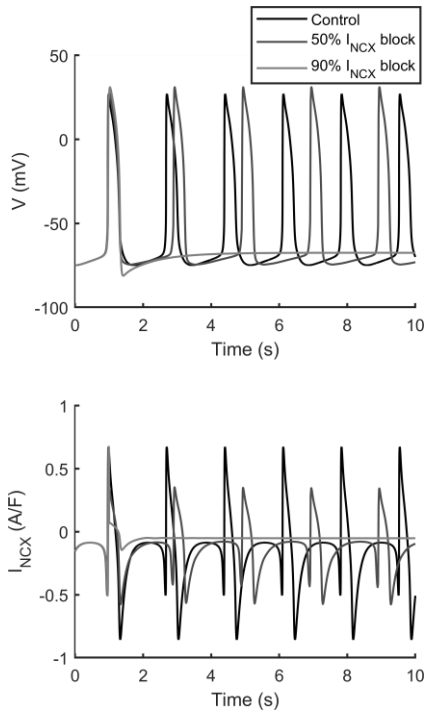

Figure 2. Effect of increasing I_{NCX} block on the Paci2019 model.

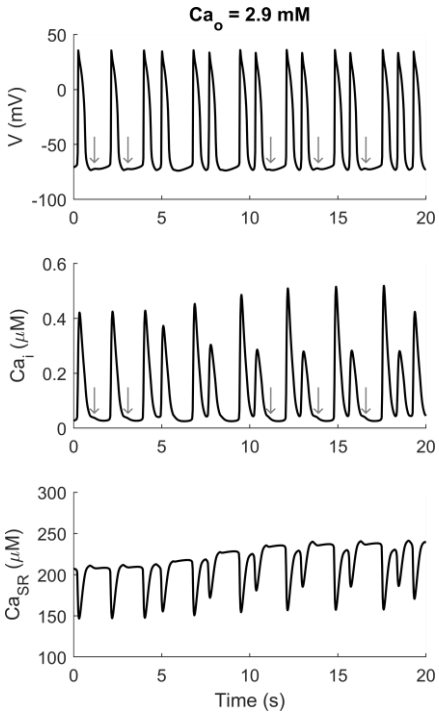

Figure 3. DADs as consequence of Ca^{2+} overload due to increased extracellular Ca^{2+} concentration.

we presented an updated hiPSC-CM model that summarizes both the mechanisms for the AP automaticity and that can be used to attempt to verify these hypotheses.

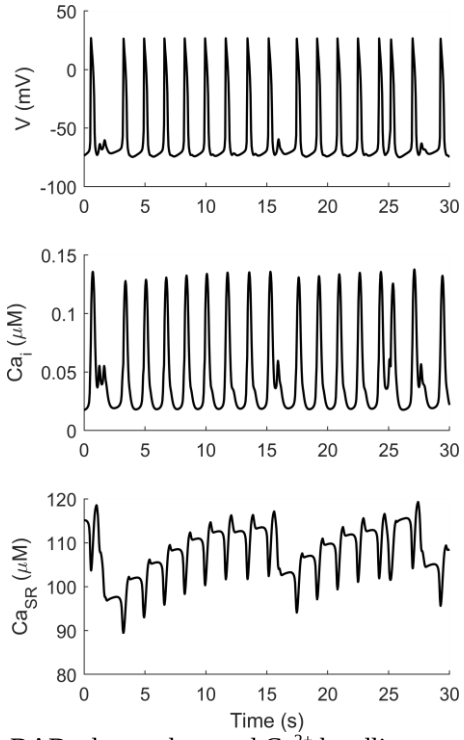

Figure 4. DADs due to abnormal Ca^{2+} handling.

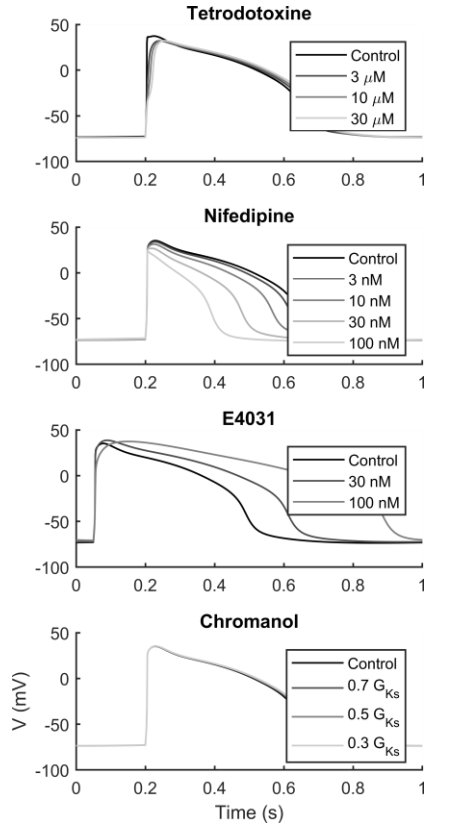

Figure 5. Model responses to current blockers.

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