Late Sodium Current Inhibition Counteracts Pro-arrhythmic Mechanisms in Human Hypertrophic Cardiomyopathy

Elisa Passini[1,2], Blanca Rodriguez[2], Ana Mincholé[2], Raffaele Coppini[3], Elisabetta Cerbai[3], Stefano Severi[1], Alfonso Bueno-Orovio[2]

[1]Department of Electrical, Electronic and Information Engineering, University of Bologna, Italy
[2]Department of Computer Science, University of Oxford, UK
[3]Department NeuroFarBa, University of Florence, Italy

Abstract

Hypertrophic cardiomyopathy (HCM) is a genetic disorder characterised by increased arrhythmic risk. The causes are still unclear, but potential pro-arrhythmic mechanisms may include increased temporal and spatial variability in action potential duration (APD) as well as repolarisation abnormalities, such as early after-depolarisations (EADs) and APD alternans.

We performed investigations of these pro-arrhythmic mechanisms and their modulation by late sodium current (I_{NaL}) inhibition, in two populations of healthy (CTRL) and HCM human endocardial action potential (AP) models, calibrated against human experimental recordings of AP and Ca^{2+} transient (CaT) in single cells.

The simulated HCM phenotype was in agreement with the experimental observations, showing prolonged AP and CaT, together with an increase in their variability. In addition, simulation results show that HCM promotes EADs and APD alternans at rapid pacing rates. Their occurrence is counteracted by I_{NaL} inhibition, suggesting this as a good therapeutic target in HCM.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is the most common monogenic cardiac disorder and the main cause of sudden cardiac death in young athletes [1]. Usually asymptomatic, it is characterised by ventricular thickening, myofibre disarray and increased arrhythmic risk. However, due to the limited understanding of the cellular mechanisms underlying the disease, a specific pharmacological treatment is still lacking.

Recently published experimental data assessed the electrophysiological profile of human HCM [2], compared with control (CTRL): diseased cardiomyocytes are characterised by prolonged action potential (AP) and Ca^{2+} transient (CaT), depending on the increase of Late Na^+ current (I_{NaL}) and L-type Ca^{2+} current (I_{CaL}), together with a decrease of K^+ repolarising currents.

Based on this experimental dataset, we constructed two populations of human cardiac AP models, to reproduce both CTRL and HCM phenotypes and to investigate *in silico* the pro-arrhythmic mechanisms potentially occurring in the disease, like early after-depolarisations (EADs) [2] and AP duration (APD) alternans [3]. In addition, since I_{NaL} current has been reported to be significantly higher in HCM (2), its selective block was studied on both populations, to evaluate the impact of this current on the pro-arrhythmic mechanisms investigated.

The population of models approach [4–6] is particularly appropriate in this context, because it provides the means to explore variability, which seems to be an important aspect in HCM and which cannot be taken into account when considering a single AP model, representative of the average cellular behaviour.

2. Methods

2.1. Experimental data

All the experimental data used in this work were collected by Coppini et al. [2], using human cardiomyocytes from n=26 HCM patients, compared with non-failing non-hypertrophic CTRL (n=8).

Single cell patch-clamp measurements and intracellular Ca^{2+} studies produced an extensive set of AP and CaT biomarkers: APD (20%, 50% and 90% of repolarisation), AP amplitude, mean upstroke velocity, mean resting potential, CaT duration (50% and 90%), CaT time to peak, CaT amplitude and diastolic Ca^{2+} concentration.

Voltage clamp on I_{to}, I_{CaL}, I_{K1} and I_{NaL} were also performed, in order to characterise electrophysiological changes occurring in the diseased condition at the ionic current level. In addition, protein studies and mRNA expression measurements were acquired, in order to quantify the changes occurring in the main ionic current subunits (I_{Kr}, I_{Ks}, I_{K1}, $I_{to,}$ I_{CaL}, I_{Na}), as well as SERCA pump, ryanodine receptors and Na^+/Ca^{2+} exchanger.

2.2. CTRL population

An initial population of 100,000 models was considered, using as baseline the O'Hara-Rudy model [7]. Some minor modifications were applied, in order to better reproduce the experimental CTRL data, i.e. an increase in the transient outward K^+ current (I_{to}) together with changes in intra- and extra-cellular ionic concentrations and K^+ reverse potential, based on the experimental protocols and I_{K1} V-clamp data.

In order to build the initial population, a total of 15 parameters, including maximal conductances of the main ionic currents and pumps/exchangers characterising the human ventricular AP, were probabilistically sampled in the [-50%, +200%] range, with respect to their baseline parameter values. The calibration process was based on AP and CaT biomarkers computed from the experimental data described above [2].

Minimum and maximum experimental values of these biomarkers, except outliers, were considered as acceptable ranges for the corresponding model biomarkers.

Based on data distribution, additional calibration constraints were included, e.g. linear correlation between APD_{50} and APD_{20}.

Only the models satisfying all the considered constraints were included in the CTRL population, resulting in about 15,000 accepted models.

Representative AP traces of accepted and discarded models are shown in Fig. 1, together with a few examples of the calibration process for some of the AP and CaT biomarkers.

2.3. HCM population

The HCM population was built from the CTRL one, by applying the electrical remodelling found in HCM ventricular myocytes [2].

Based on V-clamp experiments, we included an increase of I_{NaL} and I_{CaL} (+165% and +25%), and a decrease of I_{to} and I_{K1} (-75% and -25%).

In addition, experimental data on mRNA expressions were used to modulate the K^+ repolarizing currents (-25% I_{Kr} and -40% I_{Ks}), the SERCA pump (-15%), the ryanodine receptors release (-15%) and the Na^+/Ca^{2+} exchanger (+25%).

Finally, Ca^{2+}-calmodulin kinase II activity was upscaled to reproduce the increased phosphorylation observed in the experiments (+20%).

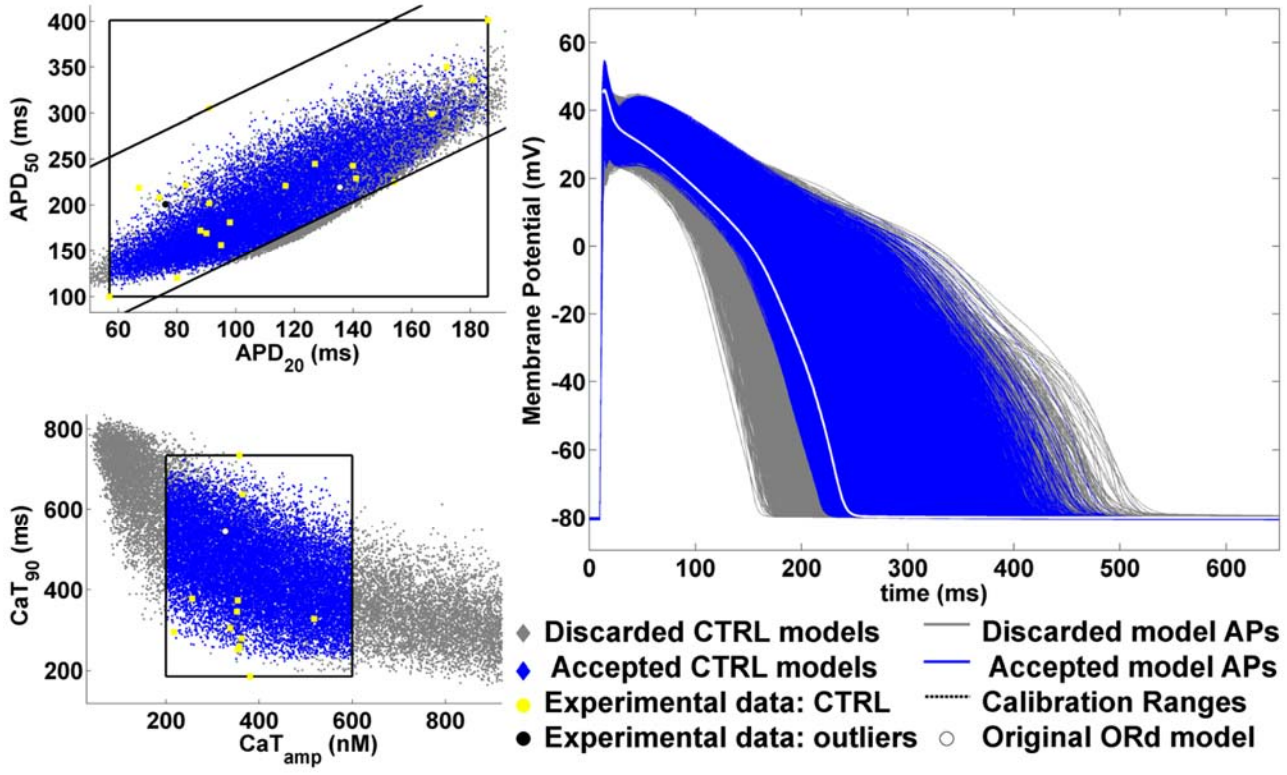

Figure 1. Experimentally-based calibration for the CTRL population of models: for the initial population (100,000 models) a complete set of biomarkers characterising AP and CaT was computed. Only those models completely in agreement with the experiments were accepted in the CTRL population.

2.4. Late sodium current inhibition

Selective I_{NaL} inhibition was applied to both populations, reducing the current maximal conductance by 60%, in accordance with the I_{NaL} V-clamp experiments recorded in presence of Ranolazine 10 μM [2].

2.5. Simulations

All numerical simulations were performed with the open source cardiac simulation software Chaste [8], using a CellML [9] implementation of the O'Hara-Rudy model, modified as described in the CTRL population methods section. All models were paced at 1 Hz until steady state (300 s). Post-processing of AP and intracellular CaT traces for biomarkers evaluation was performed within the Chaste environment, while data analysis was performed in Matlab (The Mathworks, Inc.).

3. Results

3.1. HCM phenotype

In agreement with experiments, APD was significantly longer in HCM than in CTRL, and exhibited higher variability (427±88 vs 278±49 ms, $p<0.001$). Moreover, the HCM population showed an increased Ca^{2+} transient duration, a smaller Ca^{2+} transient peak and an increase in diastolic Ca^{2+} concentration, all consistent with the HCM phenotype. APD and its variability in HCM were reduced by I_{NaL} inhibition (367±67 ms), while only a minor effect was observed in CTRL (262±47 ms) as shown in Fig. 2A.

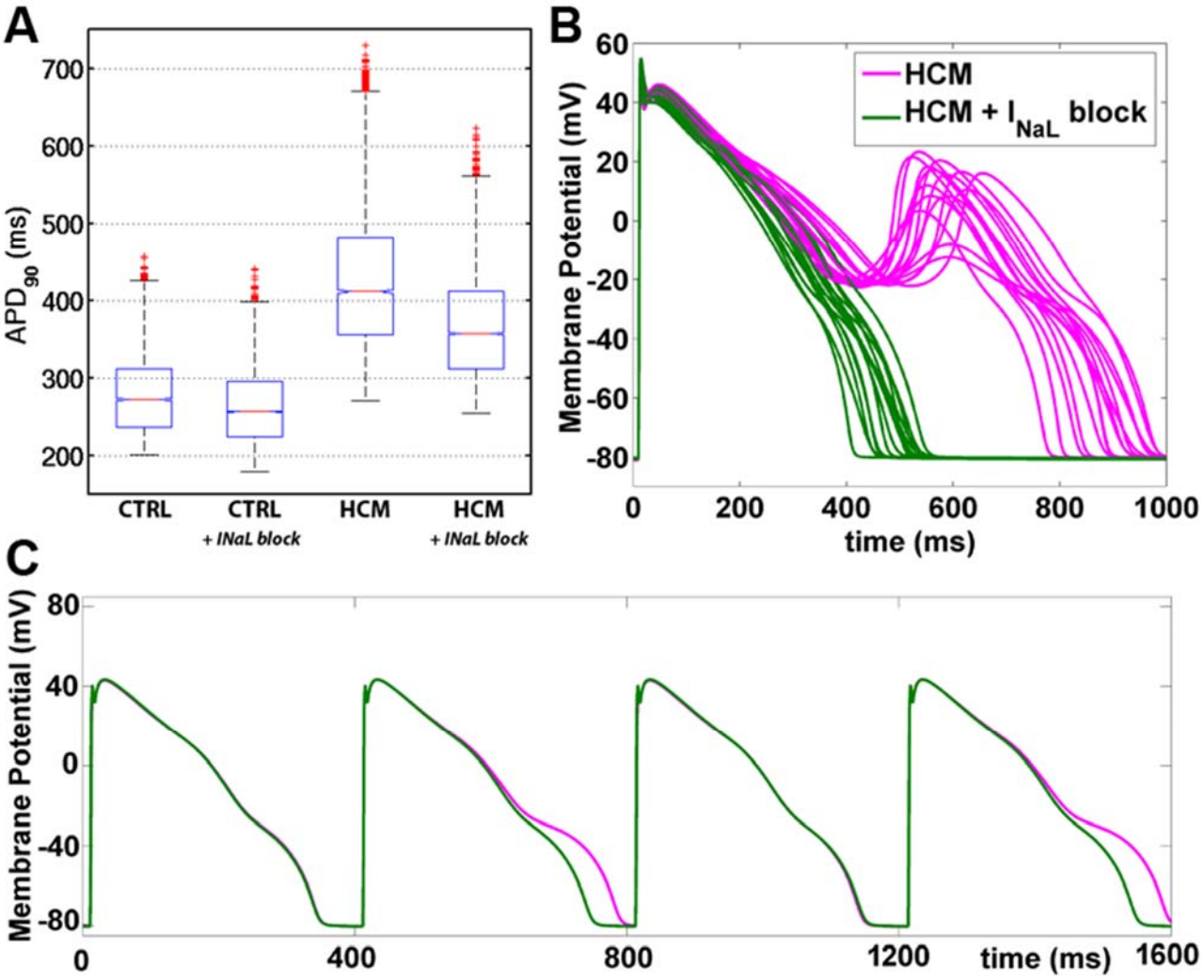

Figure 2. A) APD_{90} for the CTRL and HCM populations: selective I_{NaL} block has only a slight effect on CTRL while it significantly decreases APD_{90} in HCM. B) Representative AP traces for HCM models showing EADs, all suppressed when including I_{NaL} block. C) AP traces of one HCM model showing APD alternans at fast pacing (2.5 Hz).

3.2. EADs occurrence

Spontaneous EADs were found in about 3.5% of the HCM models. Almost all of them (97%) were suppressed when including I_{NaL} block. Some representative AP traces of HCM models showing EADs are presented in Fig. 2B.

3.3. APD alternans occurrence

Incidence of APD alternans at fast pacing rates (2.5 Hz) was significantly higher in the HCM population compared to CTRL (15% vs 3% respectively), yielding a pronounced beat-to-beat variability in APD dispersion (APD alternans magnitude of 16 [6-44] ms; values representing median and [25^{th}-75^{th}] distribution percentiles). I_{NaL} block successfully suppressed alternating behaviour in 30% of the models, and additionally decreased alternans magnitude in 35% of the remaining ones. A representative AP trace of a HCM model showing alternans is presented in Fig. 2C.

4. Discussion and conclusions

Computational simulations performed in two experimentally-calibrated populations of human ventricular models, reproducing CTRL and HCM phenotypes respectively, suggest that HCM promotes EADs and APD alternans at rapid rates.

Selective I_{NaL} inhibition seems to counteract both these pro-arrhythmic mechanisms, partially reversing the electrophysiological remodelling occurring in HCM, and therefore suggesting I_{NaL} as a good therapeutic target in HCM. Moreover, within HCM population, EADs and APD alternans occurred in models with larger I_{NaL} conductance. This emphasises the major role played by this current in the HCM phenotype, thus explaining both the higher incidence of APD alternans and the presence of abnormalities in repolarisation reserve in HCM vs CTRL, as well as their suppression by I_{NaL} inhibition.

The higher incidence of EADs in HCM vs CTRL and their decrease when considering I_{NaL} block are in agreement with the considered experimental data [2], while alternans occurrence has not been previously analysed in this disease. However, at whole organ level, HCM has also been related to T wave alternans [3], that may depend on APD alternans at cellular level.

Both EADs and APD alternans are important pro-arrhythmic mechanisms and a better understanding of their occurrence in HCM may guide the development of a disease specific treatment to reduce the arrhythmic risk of this disease.

Future work will address a better refinement of both CTRL and HCM populations, in order to further improve the HCM phenotype, thus achieving a more complete knowledge of the electrical remodelling occurring in this pathology and of the ionic mechanisms involved.

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Address for correspondence.

Elisa Passini
Computational Physiopathology Unit
Department of Electrical, Electronic and
Information Engineering "Guglielmo Marconi"
University of Bologna - Cesena Campus
Via Venezia 52, 47521 Cesena (FC), Italy
elisa.passini@unibo.it