Effect of Inter-Subject Variability in Determining Response to IKr Block in Human Ventricular Myocytes

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Abstract

Causes and impact of inter-subject variability in cellular electrophysiological behaviour are unknown. Understanding the effects of this variability is important, particularly as it can modulate response to drug application. Differences between individuals in response to drug action may be due to ionic-level differences, but the effects of these differences may be masked under normal physiological conditions. Therefore, investigating these differences is important to understand the effects of drug action on cells from different individuals.

We have developed a methodology to study variability between individuals in cardiac electrophysiology, using populations of models. We performed a simulation study to analyse the response of a population of human ventricular models calibrated using action potential recordings from control experiments, to simulated application of dofetilide, a selective blocker of the delayed rectifier potassium current (IKr). Analysis of the differences between models that developed repolarisation abnormalities and those that did not showed that L-type calcium current conductance was significantly higher and sodium-potassium pump permeability significantly lower in those models that displayed repolarisation abnormalities. Our results show that IKr block can reveal differences in susceptibility to repolarisation abnormalities between cells that appear equally healthy under control conditions.

1. Introduction

Biological variability between individuals modulates the physiological function of the heart and other organs, at all scales. It can drastically affect the outcome of exposure to pathological conditions and pharmacological intervention. In cardiac muscle, cells taken from the same species and the same location but from different hearts display qualitatively similar responses to a stimulus - an action potential. However, significant quantitative inter-subject differences can exist between these action potentials in terms of shape, duration, and dynamics.

Understanding the mechanisms underlying this variability is important, particularly as it can modulate response to drug application. Differences between individuals in response to drug action may be due to differences at the level of individual ionic currents, but the effects of these differences may not be observable under normal physiological conditions, given the large range of variation seen across action potentials from human cardiomyocytes.

For this study we investigated the effects of inter-subject variability on response of human ventricular cells to selective block of the delayed rectifier potassium current (IKr), a particularly important single channel block due to IKr's importance in repolarisation. To integrate the variability seen in our experimental data into our simulation study, we applied a populations of model methodology [1]. This methodology integrates simulations and experimental data to connect the variability observed in experimental recordings at the action potential level with hypothesised variation at the level of individual ionic currents, due to channel number variability, and other potential sources of variability between individuals.

The results of this study were that the cell models in our population that displayed repolarisation abnormalities when exposed to high levels of IKr block had increased L-type calcium conductance, and decreased sodium-potassium pump permeability, compared to models that did not display any repolarization abnormalities.

2. Methods

2.1. Experimental data

The experimental data used in this study consisted of micro-electrode recordings of right ventricular endocardial tissue samples (n=90, from 62 unique hearts) taken from non-failing human hearts, paced at 1 Hz in control conditions.

2.2. Construction of population of models

In order to study inter-subject variability in human ventricular electrophysiology, we used a population of models methodology [1]. To create the population of models used in this study, we first identified the baseline cardiac cell model to use, in this case the O'Hara-Virag-Varro-Rudy (ORd) human ventricular cell model [2]. This model was chosen because it is a biophysically detailed and well-validated model of human ventricular myocytes, and because it was parameterized primarily with data from the same laboratory as the data used in this study.

After selecting the baseline model, we then identified the parameters to vary between models. We chose 9 conductances/permeabilities in the model: GNa (fast sodium channel conductance), GNaL (late sodium channel conductance), Gto (transient outward potassium channel conductance), GKs and GKr (conductances of the slow and rapid components of the delayed potassium rectifier current), GK1 (potassium inward rectifier channel conductance), GNCX (sodium-calcium exchanger permeability), GNaK (sodium-potassium pump permeability), and GCaL (L-type calcium channel conductance). These are the conductances and permeabilities of the main ionic currents in the model. We chose to vary these parameters because we hypothesize that the numbers of different types of ion channel will vary between individuals, while the structure and kinetics of each individual ion channel type will be conserved across individuals of the same species.

Following this, we generated a large number of parameter sets by scaling these 9 conductances simultaneously across a range of +/-100% of their baseline values. Parameter sets are generated using Latin Hypercube Sampling [3], so that each parameter was sampled evenly over the parameter space.

The behaviour of these models was then simulated. To match our experimental data, we paced each model at 1 Hz, under control conditions. Additionally, we used a biphasic stimulus as in Livshitz et al. [6], as the experimental data used in this study comes from small tissue preparations. The aim of using this biphasic stimulus was to simulate the effects of surrounding cells in the preparation acting as current sinks for the stimulus current.

Following simulation, we then characterised each model's behavior from its action potential trace using 7 biomarkers: peak membrane potential, time at which peak membrane potential occurred, action potential duration (APD) at 40%, 50% and 90% of repolarisation (APD40, APD50, APD90), Triangulation (APD90 – APD40) and resting membrane potential.

To determine which of the models were consistent with experiments, we compared these biomarker values to those from experiment. For each of the 7 biomarkers mentioned above, we estimate the biomarker's physiological range – the minimum and maximum value of the biomarker seen in our experimental dataset. Only models that had all biomarker values within the ranges seen across the data were accepted into the population, and models with one or more biomarkers out of range were discarded.

The resulting population of models contains models that all have different conductance values for their individual ionic currents, which results in quantitatively different action potential behavior between models, but all of the models in the population produce action potentials that are consistent with the range of variability seen in experimental data.

2.3. Simulation of drug action

We simulated the application of 50 nM dofetilide, by modelling the drug as a single-pore IKr block [4], with an IC50 of 12 nM, and Hill coefficient of 1.7 [5]. This is equivalent to a 92% reduction of IKr.

3. Results

Using the methodology described above, we created a populations of models, using an initial pool of 10,000 models. The population was developed using control data and control conditions simulations for calibration of the population. Models that produced biomarker values within the ranges observed in control experiments, for all 7 biomarkers, were accepted into the population, and the remainder were rejected. This control conditions population contained 433 accepted models.

We then analysed the response of the population to IKr block and looked for abnormalities in repolarisation. We tested for two forms of repolarisation abnormalities: alternans (APD90 differed by 5 ms or more between the last 2 simulated beats), and after-depolarisations (any positive membrane potential/time gradient > 0.02 mV/ms after the first 100 ms of either of the last two simulated action potentials). 74 out of 433 models displayed at least one of these forms of repolarisation abnormalities. Examples of models from the population with normal and abnormal action potentials after IKr block was applied are shown in Figure 1.

Analysing the distribution of parameter values for models that displayed abnormalities (Figure 2), revealed that across the models that displayed repolarisation abnormalities, the L-type calcium current conductance (GCaL) was significantly higher, and the sodium-potassium pump permeability (GNaK) was significantly lower, compared to the models in the population that did not display repolarisation abnormalities. In the sub-population of models displaying repolarisation abnormalities, the average scaling value for GCaL was 1.07, compared to 0.37 for the sub-population with no abnormalities, while for GNaK the average scaling value

was 0.27 for the abnormality sub-population and 0.86 for the no abnormality sub-population (a scaling value of 1.0 would be equal to the original value of the conductance/permeability in the baseline ORd model).

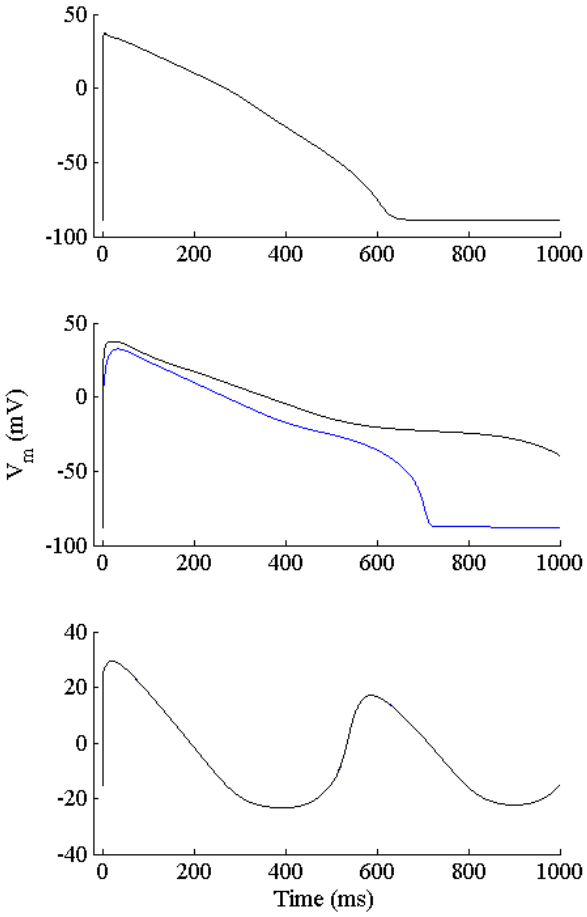

Figure 1. Examples of action potential traces from 3 different models in the population of models, with 92% IKr block at 1 Hz pacing. Models display: a regular action potential (top); alternans (middle, last two beats of simulation shown); and triggered activity (bottom).

4. Discussion and conclusions

In this study we have investigated how inter-subject variability at the ionic current level can affect the electrophysiological response of human ventricular myocytes to IKr block. Our results indicate that in cells where L-type calcium current is elevated and/or sodium-potassium pump current is reduced, inhibition of IKr can lead to repolarisation abnormalities in cells that appear healthy under normal physiological conditions. These results are consistent with the known role of the L-type calcium current in generating EADs in conditions where repolarisation is delayed [7], and with the importance of the sodium-potassium pump current in regulating cardiac repolarisation [8].

A limitation of this study is that we only considered data acquired from 1 Hz pacing. This means that rate dependent effects are not considered by our populations of models. Future work on this study will include an analysis of the behavior of the population of models under different pacing rates.

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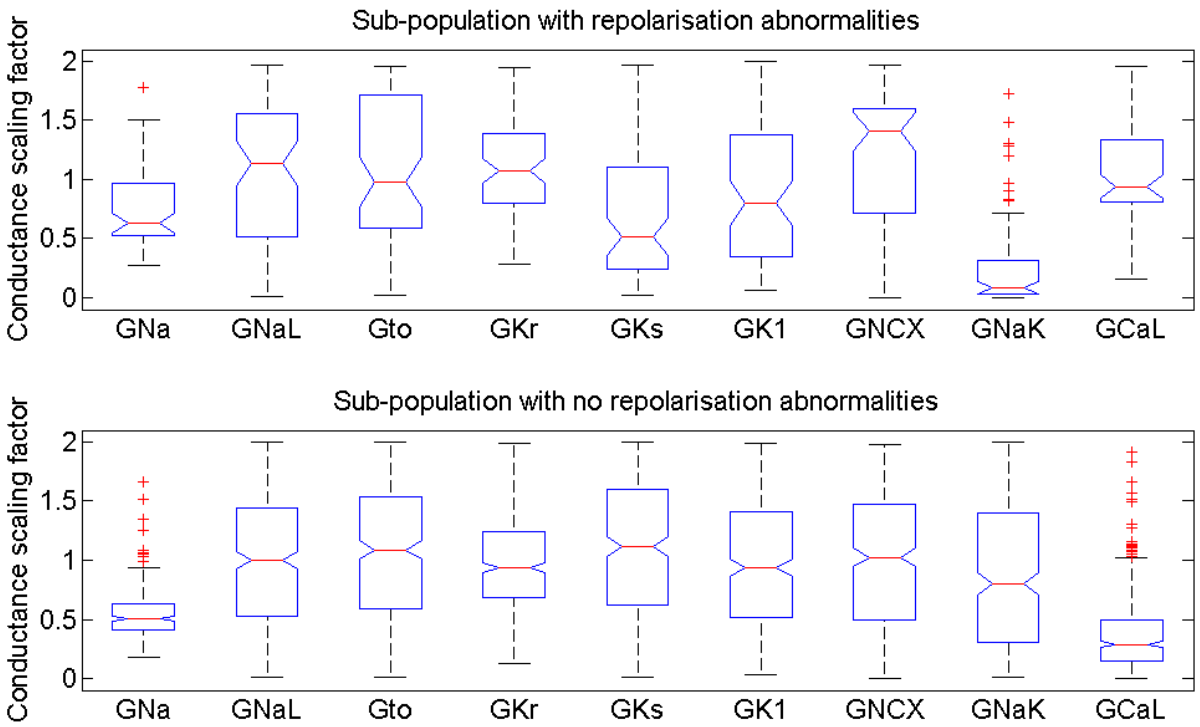

Figure 2. Parameter distributions of the models that displayed repolarisation abnormalities in response to IKr block (top) and of the models that did not (bottom). Of particular note, the magnitude of GCaL is larger and the magnitude of GNaK is smaller in the group of models with repolarisation abnormalities.