I_{kr} Impact on Repolarization and its Variability assessed by Dynamic-Clamp

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Abstract

In previous work we have shown that action potential duration (APD) and its response to modulation are "intrinsically" proportional. Nevertheless, loss of the rapid delayed rectifier K^+ current (I_{Kr}) increases APD short-term variability (BVR) beyond what expected by such proportionality. It is unclear whether this may be explained by known I_{Kr} gating properties and which among them prevails in limiting BVR. As a preliminary approach to the problem, we investigated whether: 1) BVR changes caused by I_{Kr} blockade can be reversed by injection of current generated by a deterministic numerical I_{Kr} model (mI_{Kr}); 2) modulation of I_{Kr} maximal conductance (g_{max}) may affect APD and BVR differentially. In guinea-pig myocytes, native I_{Kr} was blocked by E4031 and replaced by mI_{Kr} by Dynamic-Clamp (DC); 2) the effect on APD and BVR of relatively small changes in mI_{Kr} g_{max} were assessed. The results thus far indicate that: 1) mI_{Kr} effectively reversed E4031 effects on both APD and BVR; 2) a 30% decrease in g_{max}, inadequate to prolong APD, increased BVR significantly. We conclude that 1) the effect of I_{Kr} changes on BVR may exceed that expected from modulation of APD only; 2) this can be accounted for by the known channel gating features implemented in mI_{Kr}.

1. Introduction

During the electrical cycle, a close feed-back exists between membrane current, voltage and intracellular Ca^{2+} dynamics [1]. Therefore, the effect of an individual current perturbation on action potential ultimately depends on global system properties. Channel gating properties are generally assessed by patch-clamp studies on channel proteins expressed in heterologous cell systems. In most cases the expression system cannot generate an action potential and, in all cases, it provides an environment remote from that of a mature cardiac myocyte [2]. This makes accurate prediction of the effect of gating abnormalities on action potential stability a daunting challenge [3].

Dynamic clamp (DC) affords a unique possibility in this direction. This innovative "hybrid" technique allows to replace a native current with a modeled one in a real myocyte, whose electrical activity depends, for everything else, on myocyte own properties. In a nutshell: membrane potential is recorded from a real myocyte and fed to the computer to drive the current numerical model. The model-generated current is then passed back to the myocyte, thus affecting the action potential in a virtually real-time loop [4]. Gating abnormalities can be easily implemented in the current model and their impact on electrical activity can be tested by replacing the native myocyte current, suitably blocked, by Dynamic Clamp.

Beat-to-beat variability of action potential duration (BVR), is an index of electrical instability and a strong predictor of pro-arrhythmia in several experimental models [5,6]. In a single myocyte, BVR is generated by stochastic current variability, which can be either buffered or amplified by the deterministic system feed-back network.

Dynamic Clamp has particular appeal in testing BVR mechanisms, because it introduces a fully deterministic current description (the model) in the context of global system behaviour, including its stochastic and complexly interacting deterministic components [1]. Therefore, the changes in BVR resulting from modulation of model parameters, may provide information on which properties of the modeled current are more likely to increase or decrease overall system stability. This affords the possibility of a systematic "sensitivity analysis" of the impact of current parameters on electrical stability. Provided that the current model used in the process is adequate, this may assist in linking current gating properties to cellular electrical stability. This is instrumental to the development of specific therapeutic approaches to arrhythmogenic channelopathies and to the prediction of drug-induced proarrhythmia.

The aim of this work is to explore Dynamic Clamp performance in 1) testing the adequacy of a numerical I_{Kr}

model in replacing native I_{Kr} for what concerns APD and BVR modulation; 2) carrying out preliminary analysis of APD and BVR sensitivity to changes in I_{Kr} maximal conductance.

2. Methods

This investigation conforms to the Guide to the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and to the guidelines for Animal Care endorsed by the University of Milan.

Experiments were performed on freshly isolated guinea-pig ventricular myocytes at 36.5 °C. Action potentials (APs) were recorded by patch clamp (I-Clamp mode, IC) in the whole-cell configuration. Myocytes were superfused at 2 ml/min with Tyrode's solution containing 154 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES-NaOH, and 5.5 mM d-glucose, adjusted to pH 7.35. The pipette solution contained 110 mM K^+-aspartate, 23 mM KCl, 0.2 mM $CaCl_2$ (calculated free $Ca^{2+} = 10^{-7}$ M), 3 mM $MgCl_2$, 5 mM HEPES KOH, 0.5 mM EGTA KOH, 0.4 mM GTP-Na salt, 5 mM ATP-Na salt, and 5 mM creatine phosphate Na salt, pH 7.3. Specific I_{Kr} blocker (E4031 5 µM) was added to Tyrode solution.

The profile of the current sensitive to the I_{Kr} blocker E4031 5 µM (I_{E4031}, representative of I_{Kr}) during the action potential was recorded in several cells at CL = 1000 ms by clamping membrane voltage with action potential waveforms of the same myocytes (action-potential clamp, AP-clamp) [7]. An average I_{E4031} profile thus obtained was used as the template to develop a numerical model of I_{Kr} (mI_{Kr}), based on optimization of the Luo-Rudy (LRd) formulation [8]

BVR was calculated from 40 consecutive beats at CL = 1000 ms according to [9]

$$BVR = \Sigma(|APD90n+1 - APD90n|)/[nbeats \times \sqrt{2}]$$

Where APD90 is APD at 90% repolarization , n is beat number and nbeats the total number of beats

Dynamic-clamp

Native myocyte I_{Kr}, was blocked by E4031 5 µM and replaced with mI_{Kr} at cycle length (CL) of 1000 ms. APD and its BVR were compared within the same myocyte between control (ctr), I_{Kr} blockade (E4031) and after its replacement with mI_{Kr} (E4031+mI_{Kr}).

The maximal conductance (g_{max}) of mI_{Kr}, was symmetrically changed around its baseline value (0.032 nS) by ± 0.010 nS. APD and BVR were measured under dynamic-clamp with the baseline g_{max} and its ± 0.010 nS values.

3. Results

I_{Kr} replacement with mI_{Kr}

Even of slightly larger in amplitude, mI_{Kr} accurately reproduced I_{E4031} profile during the action potential (Fig 1 right). APD and BVR were increased by exposure to E4031; the effect of E4031 on both APD and BVR was completely reversed by mI_{Kr} injection in dynamic-clamp mode (Fig 1 right).

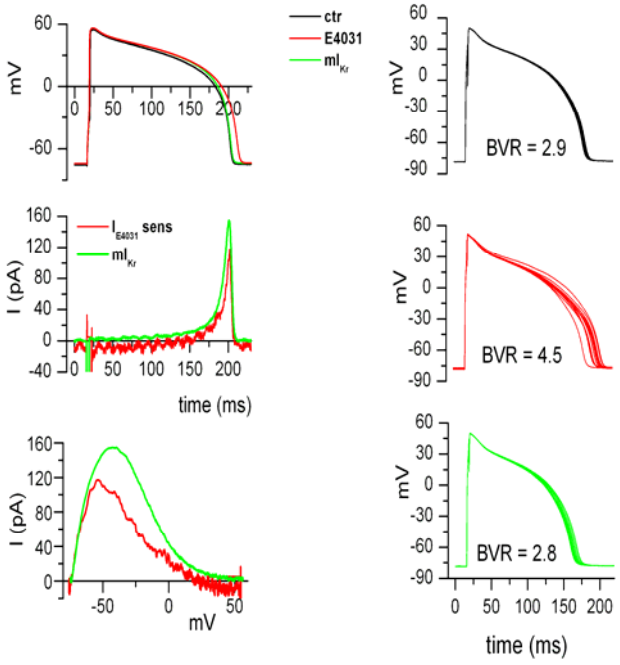

Figure 1. Left: mI_{Kr} (green) is compared to I_{E4031} recorded under AP-clamp conditions (red) from the same myocyte (top: action potential, middle I_{E4031} and mI_{Kr} profiles; bottom: dynamic I/V relationships for the two currents. Right: 10 subsequent APs recorded from the same myocyte in control (black), during exposure to E4031 (red) and during I_{Kr} replacement (E4031+mI_{Kr}) (green).

Effect of changes in mI_{Kr} maximal conductance (g_{max})

An illustrative example of change in g_{max} is shown in Fig 2. A decrease in g_{max} increased BVR (from 2.8 to 3.7 ms) without significantly prolonging APD (from 181.2 to 187.7 ms). An increase in g_{max} (Δ = + 0.010 nS) did not affect BVR (2.9 ms) and slightly shortened APD.

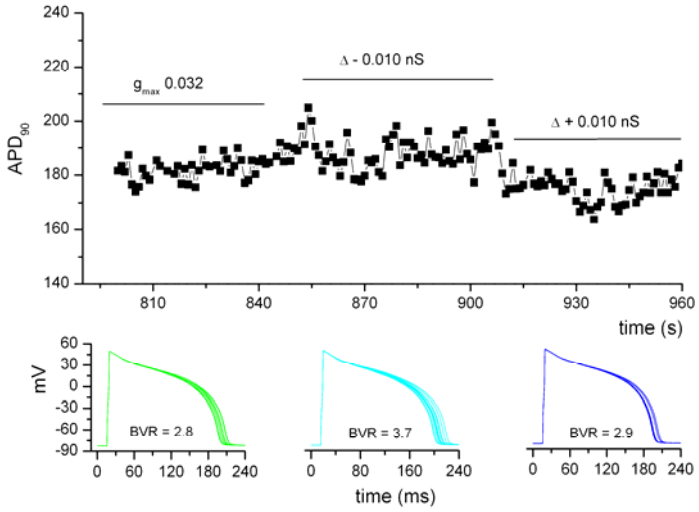

Figure 2. Top: representative time-series of APD values recorded at three g_{max} values (bars). Bottom: 10 consecutive action potentials and BVR value for each g_{max} value (aligned with the the top panel).

4. Discussion

The results obtained indicate that in a cardiac myocyte the effect of I_{Kr} blockade on APD and BVR can be simultaneously reversed by the injection of modeled I_{Kr}. This finding indicates that known I_{Kr} properties, implemented in the model, are sufficient to account for I_{Kr} role in modulation of both action potential duration and its stability at the same time. While of importance as such, this evidence would still be compatible with BVR being intrinsically dependent on APD, a property previously described. Nevertheless, the present results also indicate that changes in maximal I_{Kr} conductance exerting minimal effects on APD may significantly affect BVR. This implies that I_{Kr} properties, implemented in the numerical model, can modulate BVR independently of APD. This is likely the result of feed-back I_{Kr} interaction with "global" system properties relevant to repolarization stability. While further analysis is required to assess the chain of events involved in the feed-back, the present result identifies g_{max} as a "hot" parameter in BVR modulation. Studies will be continued to provide a systematic analysis of APD and BVR sensitivity to changes in all I_{Kr} gating parameters. The result of such an endeavor may conceivably assist in predicting the impact of a given gating abnormality, previously identified by patch-clamp in heterologous cell systems, on electrical stability of the native cardiomyocyte. In a translational perspective, ultimate validation of such predictions might be achieved by comparison with available clinical data linking I_{Kr} mutations to their phenotypic expressivity.

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