Divergent Action Potential Morphology in Human Atrial Cells compared with Tissue: Underlying Ionic Mechanisms

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Abstract

This study aimed to elucidate the mechanisms underlying the divergent action potential (AP) morphology observed in human atrial cells vs. tissue, with simulations employing computational models.

Two modifications were introduced to the cell model, based on conditions inherent to in vitro AP measurements. First, we accounted for the loss of hERG ion channels, which mediate the rapid delayed rectifier potassium current (I_{Kr}), due to the standard enzymatic cell isolation protocol. Second, the effect of adding a intracellular calcium buffer (EGTA), which is typically used in patch clamp measurements of APs, was also considered.

The reduced I_{Kr} conductance slowed repolarization (AP phases 2 and 3) significantly, while initial repolarization (AP phase 1) remained largely unaffected. Whereas, addition of the EGTA buffer in silico affected both the initial and later phases AP repolarization. Simulation results show that changes in AP morphology depend rather dramatically on the chosen mathematical formulation of calcium and potassium currents. Accordingly, it is important to consider this divergence in electrophysiological properties when, for example, extrapolating pharmacological effects simulated or measured in single cells to intact cardiac tissue.

1. Introduction

The diversity of action potential (AP) morphology in human atrial myocardium was first reported *in vitro* by Gelband et al. [1], who assigned two different categories for AP types: specialized and contractile fibers. This kind of principal division corresponds to both early anatomical studies [2] and what is currently known of the underlying molecular basis of heterogeneity [3]. In atrial cells isolated from tissue, three categories have been used to describe AP shape [4,5], and they have been linked, for example, to the cell-specific magnitudes of transient and sustained outward potassium currents (Ito and I_{sus}/I_{Kur}).

Adding to this complexity, the enzymes that are commonly used to isolate cells from human cardiac tissue have been reported to damage the hERG channel protein, resulting in a loss of rapid delayed rectifier potassium current, I_{Kr} [6,7]. Furthermore, addition of intracellular calcium buffering (typically EGTA) is a standard procedure in single cell patch clamp measurements of APs to stabilize conditions, i.e. to suppress mechanical activity.

It is a common *in vitro* finding that AP shape tends to be of the spike-and-dome variety or have a more pronounced plateau in tissue measurements, whereas a more triangular AP shape is typically reported in isolated myocytes (Figure 1). To what extent these observations are affected by loss of I_{Kr} and extra calcium buffering is not well understood. The aim of this study to analyze those two perturbations with respect to their effect on AP morphology in computational cell models.

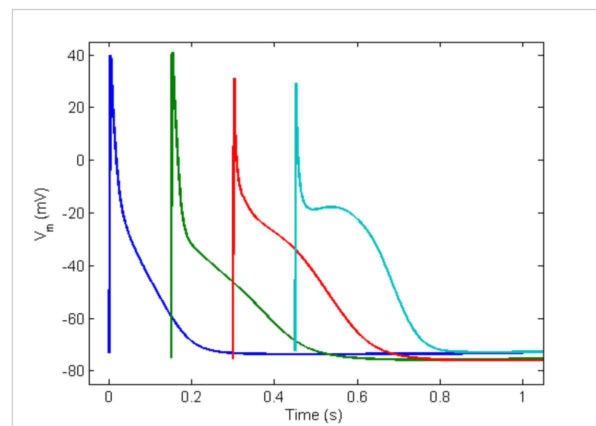

Figure 1. Examples of human atrial AP morphology.

2. Methods

Our recently published human atrial cell model, which incorporates a physiologically-based description of intracellular calcium dynamics [8], will serve as modeling platform. Two model variants are used in this study: 1) original, and 2) original + modified L-type calcium current (ICaL) and potassium current formulations according to Courtemanche et al. [9]. We refer to these model variants as "orig" and "*mod*" in the following text. The only modification to the Courtemanche et al. current formulations is decreased conductance (5.7 vs. 9.0 nS) of the inward rectifier current I_{K1}, to match the resting membrane potential (~ -76mV) of the model variants.

Using the two model variants, we assess the effect of I_{Kr} loss (conductance reduced to one sixth of control) and addition of calcium buffer (EGTA 5mM, K_d = 0.12 μM) in a single cell environment. The effect of these two modifications are measured *in silico* at quasi steady-state (5 minutes of pacing = 300 cycles, starting from a quiescent steady-state) at a basic cycle length of 1000 ms. Stimulus current amplitude was set to 1.5-fold the threshold unless stated otherwise.

3. Results

3.1. AP shape in the two model variants

The formulation of potassium currents of the two model variants differ substantially. In the *orig* model, repolarization of AP relies mainly on I_{sus}, which is common to the Nygren pedigree of human atrial models. In the *mod* model, on the other hand, I_{Kr} has a much more decisive role for AP duration (APD). Consequently, the *mod* model has a much less negative plateau potential and APD at 75% repolarization (APD75) is 50% larger, as illustrated in Figure 2. In contrast, the initial repolarization is much faster (APD75 is 57% smaller) in the *mod* model than in the *orig* model, due to larger I_{to}.

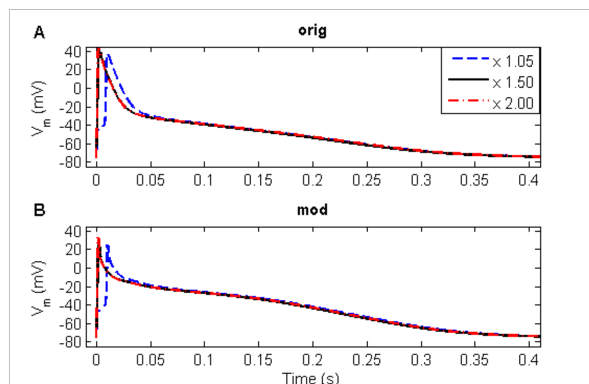

Figure 2. AP morphology of the two model variants and effect of stimulus current amplitude (x-fold threshold).

In order to compare the model variants in matching conditions, we calculated the thresholds for stimulus current amplitude and further studied its effect on AP shape. As shown Figure 2, there is some delay in the AP upstroke, if the stimulus is just above threshold, whereas increasing the amplitude from 1.5-fold to 2-fold has no significant effect on the AP shape.

3.2. Loss of I_{Kr} lengthens the AP

The effect of I_{Kr} loss due to enzymatic cell isolation on simulated cell function is shown in Figure 3 and summarized in Table 1. Loss of IKr causes a dramatic lengthening of AP (Figure 3B). Secondarily, this results in a pronounced slowing of inactivation of I_{CaL} (Figure 3D; calcium influx, J_{CaL} +32%) and increased activation of I_{Ks} (Figure 3F) in the *mod* model, whereas the effects in the *orig* model are comparatively small (Figure 3 A, C and E).

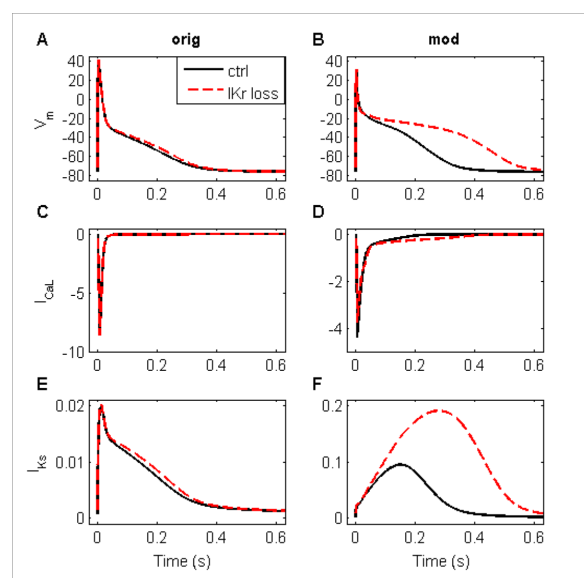

Figure 3. Effect of I_{Kr} loss on the AP shape (A, B), I_{CaL} (C, D) and I_{Ks} (E, F) in the two model variants.

Table 1. Change in APD due to I_{Kr} loss in the two model variants.

	orig	*mod*
ΔAPD_{20}	~0	–6%
ΔAPD_{75}	+15%	+90%
ΔAPD_{90}	+8%	+68%

3.3. Calcium buffering further modifies AP shape

The effect of combined IKr loss and added calcium buffering (EGTA) on simulated cell function is shown in Figure 4 and summarized in Table 2. Added calcium buffering changes both the initial and late phase of AP repolarization. These changes are caused by altered ICaL (Figure 4C and D) and I_{NCX} (Figure 4 E and F) via changed calcium-dependent inactivation of ICaL, due to blunted calcium transient in the subsarcolemmal space (Figure 4G and H). Strikingly, the two model variants predict opposing changes in AP morphology (Table 2).

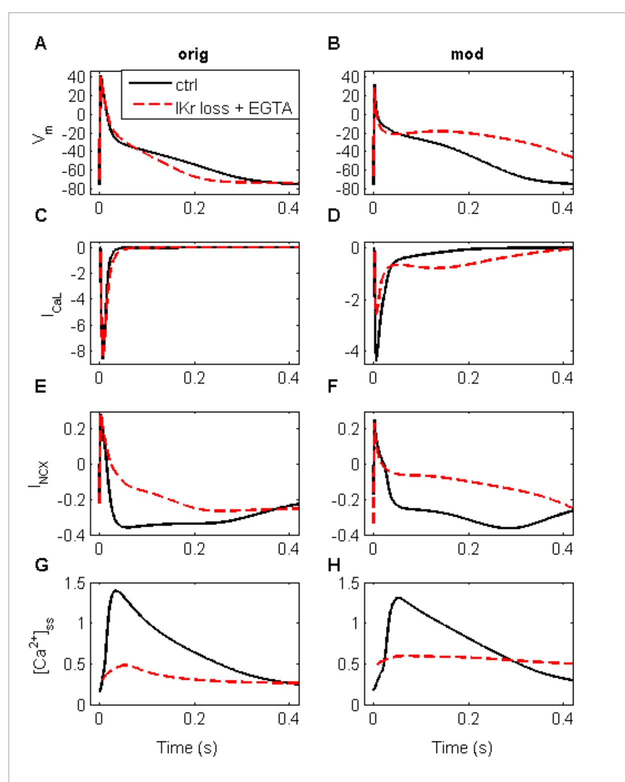

Figure 4. Effect of combined I_{Kr} loss and added calcium buffering on the AP shape (A, B), I_{CaL} (C, D), I_{NCX} (E, F) and calcium transient in the subsarcolemmal space (G, H) in the two model variants.

Table 2. Change in APD due to combination of I_{Kr} loss and calcium buffering in the two model variants.

	orig	mod
ΔAPD_{20}	+2%	–18%
ΔAPD_{75}	–28%	+87%
ΔAPD_{90}	–33%	+69%

4. Conclusions

Predictions of the two model variants are widely variable. First, the extent of AP lengthening due to loss of I_{Kr} is roughly 7-fold larger in the *mod* model. Second, inclusion of EGTA calcium buffer induces opposite effects in the initial and late repolarization (Table 2) in the two model variants.

The *mod* model more effectively captures the pronounced plateau observed in tissue measurements, whereas the *orig* model can replicate the triangular shape of AP reported in single cell measurements.

Underlying causes for either model variant failing to reproduce findings in both single cell and tissue measurements at least partly relate to the formulation of I_{CaL}. That is, the Courtemanche-based I_{CaL} has very little voltage-dependent inactivation, because of overly large time constant, whereas the Koivumäki et al. formulation of I_{CaL} relies too much on voltage-dependent inactivation, with only a small contribution from the calcium-dependent inactivation. Future modeling work should aim to find a “middle ground” I_{CaL} formulation and possibly also readjust the relative contribution of transient, sustained and delayed potassium currents to repolarization to provide a better match between *in silico* and *in vitro* results.

We conclude that both loss of I_{Kr} and added calcium buffering (EGTA) have a significant effect on simulated AP morphology. It is important to consider this divergence in electrophysiological properties when, for example, extrapolating pharmacological effects simulated or measured in isolated cells to intact cardiac tissue.

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