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Atrial-selective targeting of arrhythmogenic phase-3 early afterdepolarizations in human myocytes

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ABSTRACT

Background: We have previously shown that non-equilibrium Na^+ current (I_{Na}) reactivation drives isoproterenol-induced phase-3 early afterdepolarizations (EADs) in mouse ventricular myocytes. In these cells, EAD initiation occurs secondary to potentiated sarcoplasmic reticulum Ca^{2+} release and enhanced Na^+/Ca^{2+} exchange (NCX). This can be abolished by tetrodotoxin-blockade of I_{Na}, but not ranolazine, which selectively inhibits ventricular late I_{Na}.
Aim: Since repolarization of human atrial myocytes is similar to mouse ventricular myocytes in that it is relatively rapid and potently modulated by Ca^{2+}, we investigated whether similar mechanisms can evoke EADs in human atrium. Indeed, phase-3 EADs have been shown to re-initiate atrial fibrillation (AF) during autonomic stimulation, which is a well-recognized initiator of AF.
Methods: We integrated a Markov model of I_{Na} gating in our human atrial myocyte model. To simulate experimental results, we rapidly paced this cell model at 10 Hz in the presence of 0.1 μM acetylcholine and 1 μM isoproterenol, and assessed EAD occurrence upon return to sinus rhythm (1 Hz).
Results: Cellular Ca^{2+} loading during fast pacing results in a transient period of hypercontractility after return to sinus rhythm. Here, fast repolarization and enhanced NCX facilitate I_{Na} reactivation via the canonical gating mode (i.e., not late I_{Na} burst mode), which drives EAD initiation. Simulating ranolazine administration reduces atrial peak I_{Na} and leads to faster repolarization, during which I_{Na} fails to reactivate and EADs are prevented.
Conclusions: Non-equilibrium I_{Na} reactivation can critically contribute to arrhythmias, specifically in human atrial myocytes. Ranolazine might be beneficial in this context by blocking peak (not late) atrial I_{Na}.

1. Introduction

Early afterdepolarizations (EADs) are abnormal depolarizations of cardiac myocyte membrane potential (E_m) occurring during phase-2 or phase-3 of the action potential (AP). EADs have been implicated as primary mechanisms promoting ventricular arrhythmias, including Torsades des Pointes, polymorphic ventricular tachycardia and ventricular fibrillation [1]. In ventricular cells from large mammals EADs generally occur in conditions of prolonged AP duration (APD) or reduced repolarization reserve, due either to an increase in inward currents or decrease in outward currents, which promotes L-type Ca^{2+} current (I_{Ca}) recovery from inactivation and subsequent reactivation, forming the EAD upstroke (Fig. 7A). The imbalance between inward and outward currents can be caused by direct changes in sarcolemmal ion transport, or by discoordinated Ca^{2+} handling, leading to spontaneous sarcoplasmic reticulum (SR) Ca^{2+} release and subsequent increase in inward Na^+/Ca^{2+} exchange (NCX) current (I_{NCX}), as in delayed afterdepolarizations (DADs, see Fig. 7B-C). Either way, I_{Ca} eventually carries the majority of inward charge contributing to the afterdepolarization upstroke [1].

Recently, we described a novel and unique mechanism underlying phase-3 EADs (Fig. 7D) in ventricular myocytes from arrhythmia-susceptible mice overexpressing Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [2]. EADs appearing during β-adrenergic

Abbreviations: ACh, acetylcholine; AF, atrial fibrillation; AP, action potential; APD, action potential duration; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; DAD, delayed afterdepolarization; EAD, early afterdepolarization; E_m, membrane potential; I_{Ca}, L-type Ca^{2+} current; I_{Na}, Na^+ current; I_{Kr}, delayed rectifier K^+ current; I_{NCX}, Na^+/Ca^{2+} exchanger current; ISO, isoproterenol; NCX, Na^+/Ca^{2+} exchanger; pAF, paroxysmal atrial fibrillation; PV, pulmonary vein; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; UDB, use-dependent block.

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stimulation in these mouse myocytes initiated at membrane potentials negative to −45 mV, and were thus incompatible with reactivation of I_{Ca}. These events also required intact SR Ca^{2+} release but generally occurred prior to or at the same time as the peak of the triggered Ca^{2+} transient, and thus were not driven by secondary spontaneous SR Ca^{2+} release during repolarization [2]. Computational modeling revealed that these EADs were initiated by non-equilibrium reactivation of fast Na^+ current (I_{Na}), not late I_{Na}, and were secondary to enhanced SR Ca^{2+} release and augmented I_{NCX} that held voltage at a negative late plateau [2]. This was confirmed experimentally by the observation that 10 μM ranolazine, which selectively inhibits late I_{Na} in ventricles, did not prevent EAD initiation, whereas tetrodotoxin eliminated EADs [2] at a dose (1 μM) sufficient for approximately half inhibition of peak I_{Na} and 30% inhibition of late I_{Na}. By imposing different voltage waveforms on the Na^+ channel model, we confirmed that specific E_m dynamics (e.g., rapidly repolarizing ramps) permit non-equilibrium reactivation, as opposed to an equilibrium component (e.g., window current) that would emerge after holding the voltage for a prolonged time [2].

This mechanism involving Ca^{2+}-driven non-equilibrium I_{Na} reactivation is permitted by two key characteristics of the murine AP: a triangular repolarization trajectory with short and negative plateau phase (which hastens I_{Na} recovery from inactivation), and a repolarization phase that is strongly modulated by SR Ca^{2+} release and inward I_{NCX}. Thus, phase-3 EADs driven by non-equilibrium I_{Na} gating may constitute a novel mechanism of triggered arrhythmia in cardiac cell types known to exhibit these characteristics, including human atrial myocytes. Indeed, a mechanism involving phase-3 EADs driven by SR Ca^{2+} release and I_{NCX} has been shown to initiate triggered activity and re-induce atrial fibrillation (AF) in isolated canine atria (see Fig. 7E) and pulmonary vein (PV) sleeves during combined sympathetic and parasympathetic stimulation [3–5]. An interaction of both sympathetic and parasympathetic systems is thought to underlie the initiation of clinical AF in humans [6–8] and dogs [9,10].

Here we sought to determine whether these dynamics may be relevant to the human atrium, where the relatively brief atrial AP and negative AP plateau may permit I_{Na} reactivation events. Indeed, without any parameter adjustment, we replicated phase-3 EADs in silico using our established human atrial cell model with updated I_{Na} description (see Methods section). Further, we tested the hypothesis that ranolazine, which blocks both peak and late I_{Na} in atria (as opposed to the late I_{Na} selectivity in ventricles) [11–13] and suppresses AF by prolonging the effective refractory period [14], can also prevent phase-3 EAD-mediated triggered activity.

2. Methods

We integrated a Markov formulation of I_{Na} [15,16] into our model of human atrial myocyte electrophysiology and Ca^{2+} cycling [17]. We further expanded the I_{Na} Markov model to describe the interaction of the Na^+ channel with ranolazine, using the approach introduced by the Clancy group [18,19]. Based on the assumption that the channel can reside in any state in drug-free or drug-bound conditions, the new model structure is characterized by drug-free and drug-bound layers, as shown in Fig. 1. Transitions between layers were initially modeled as done by Moreno et al. [19], and transitions within the drug-bound layer were modified relative to the drug-free layer as in [19]. Several voltage-clamp protocols were simulated for parameterization of the ranolazine-I_{Na} model at room temperature: activation, steady-state inactivation, recovery from inactivation, tonic block of peak and late I_{Na}, use-dependent block (UDB), and recovery from UDB. A detailed description of the parameter identification process can be found in [19]. In our implementation, minor parameter adjustments were required to account for the different inactivation scheme in our Markov model vs. that used in [19]. Specifically, we modified the transitions between states IF and IM_1 in the drug-bound layer to match the frequency-dependence of UDB observed in experiments. The ranolazine-dependent tonic block of the delayed rectifier K^+ current (I_{Kr}) was also integrated as in [19]. Current-clamp experiments were simulated at 37 °C (the Q_{10} for scaling I_{Na} kinetics was 2.1 [20]) to assess the occurrence of EADs with or without ranolazine in the presence of isoproterenol (ISO) and/or acetylcholine (ACh), modeled using the methods of Grandi et al. [17]. All simulations were performed in MATLAB (The MathWorks, Natick, MA, USA) using the stiff ordinary differential equation solver ode15s. The model code is available for download at the following webpage: somapp.ucdmc.ucdavis.edu/Pharmacology/bers.

3. Results

Burashnikov and Antzelevitch [5] observed the development of phase-3 EADs after spontaneous termination of AF in canine atrial tissue (Fig. 2A). Under similar conditions (ACh-abbreviated AP, in absence of sympathetic stimulation), our model exhibited a similar slowing of phase-3 repolarization (Fig. 2B, top panel) upon returning from rapid pacing (10 Hz) to sinus rhythm (1 Hz). This effect is due to pause-induced increase in SR Ca^{2+} release and, consequently, enhanced inward I_{NCX}. In fact, Ca^{2+} accumulation in the cell during the fast pacing interval resulted in a transient period of hypercontractility immediately after return to normal sinus rhythm (Fig. 2B, bottom). Increased Ca^{2+} extrusion via NCX contributes to generating the inward current responsible for AP plateau prolongation, i.e., as observed in mouse [2]. Beat by beat, SR Ca^{2+} load decreased, leading to a gradual reduction of the Ca^{2+} transient amplitude (Fig. 2C, right) and I_{NCX}, which caused progressive AP shortening (Fig. 2C, left).

Heart rate variability analysis in human patients [6–8] and nerve activity recordings in ambulatory dogs [9,10] indicated that the onset of AF is associated with simultaneous discharge of both sympathetic and parasympathetic limbs, rather than by either vagal or sympathetic activity alone. Consistently, experimental studies involving autonomic nerve stimulation [4], or exogenous agonists [3], suggest that combined sympathetic and parasympathetic challenge is capable of eliciting phase-3 EADs and triggered activity. To emulate experimental protocols, we paced our atrial cell model rapidly (10 Hz), while simulating combined administration of ACh (0.1 μM) and ISO (1 μM). Again, simulations predicted a transient increase in Ca^{2+} transient amplitude after return to sinus rhythm (1 Hz pacing) due to augmented SR Ca^{2+} loading and release (Fig. 3). This enhances I_{NCX}, which favors AP prolongation and recruits non-equilibrium I_{Na} reactivation via the canonical gating mode (O), thus leading to the development of phase-3 EADs (Fig. 3, asterisks). In Fig. S1 we confirmed that during rapid repolarization ramps total I_{Na} (Fig. S1B, black/red traces) is carried mainly by a non-equilibrium component, because this is already substantial at E_m (and times) where the theoretically maximal equilibrium (window) current of I_{Na} is virtually absent (Fig. S1C, black/red traces). These results extend our observations in murine ventricles [2], and confirm that non-equilibrium I_{Na} reactivation is a likely arrhythmia mechanism in human atrial cells.

We performed additional simulations to assess the likelihood of these EADs to occur and determine the factors that most markedly affect EAD formation. We varied ion channel conductances and maximal transport rates by +/− 3–5% and assessed the occurrence of EADs using the protocol in Fig. 3 (Table S1). As expected, we found EAD initiation to be especially sensitive to I_{Na} conductance. Changes in ACh (analyzed in 5-nM increments) or ISO doses, duration of rapid pacing (1-s increments) duration of the pause (50-ms decrements), also affect EAD occurrence by modulating the repolarization rate or I_{Na} availability. Specifically, starting from the original base-case parameter configuration, [ACh] higher than 135 nM, rapid pacing protocols longer than 23 s, or pauses shorter than 800 ms prevent EADs in the model, primarily by increasing repolarization rate, reducing I_{Na} availability, or both affecting Ca^{2+} loading and I_{Na}, respectively. While ISO effects in this

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