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Mechanism for Triggered Waves in Atrial Myocytes

Yohannes Shiferaw,[1,*] Gary L. Aistrup,[2] and J. Andrew Wasserstrom[2]
[1]Department of Physics and Astronomy, California State University, Northridge, California and [2]Department of Medicine (Cardiology) and the Feinberg Cardiovascular Research Institute, Northwestern University Feinberg School of Medicine, Chicago, Illinois

ABSTRACT Excitation-contraction coupling in atrial cells is mediated by calcium (Ca) signaling between L-type Ca channels and Ryanodine receptors that occurs mainly at the cell boundary. This unique architecture dictates essential aspects of Ca signaling under both normal and diseased conditions. In this study we apply laser scanning confocal microscopy, along with an experimentally based computational model, to understand the Ca cycling dynamics of an atrial cell subjected to rapid pacing. Our main finding is that when an atrial cell is paced under Ca overload conditions, Ca waves can then nucleate on the cell boundary and propagate to the cell interior. These propagating Ca waves are referred to as "triggered waves" because they are initiated by L-type Ca channel openings during the action potential. These excitations are distinct from spontaneous Ca waves originating from random fluctuations of Ryanodine receptor channels, and which occur after much longer waiting times. Furthermore, we argue that the onset of these triggered waves is a highly nonlinear function of the sarcoplasmic reticulum Ca load. This strong nonlinearity leads to aperiodic response of Ca at rapid pacing rates that is caused by the complex interplay between paced Ca release and triggered waves. We argue further that this feature of atrial cells leads to dynamic instabilities that may underlie atrial arrhythmias. These studies will serve as a starting point to explore the nonlinear dynamics of atrial cells and will yield insights into the trigger and maintenance of atrial fibrillation.

INTRODUCTION

Excitation-contraction (EC) coupling is mediated by Calcium (Ca) signaling where membrane-bound voltage-sensitive channels induce the release of intracellular Ca, which leads to cell contraction (1,2). The signaling between these channels occurs within thousands of dyadic junctions in the cell where a few L-type Ca channels (LCCs) are in close proximity to a cluster of Ryanodine receptors (RyRs), which control the flow of Ca sequestered within the sarcoplasmic reticulum (SR). Given the local nature of Ca signaling, the spatial distribution of dyadic junctions will determine the time course of Ca release in the cell. In cardiac cells, this distribution is dictated by the t-tubule system, which consists of tubular invaginations of the cell membrane that distribute membrane channels into the cell interior, insuring a uniform spread of excitation throughout the cell. However, the extent to which t-tubules penetrate the cell can vary substantially between cell types (3,4). In ventricular cells, t-tubules extend deep into the cell along Z planes so that Ca signaling effectively occurs within the full 3D volume of the cell. This arrangement allows for a rapid and synchronized Ca release leading to a fast coordinated contraction. However, in atrial cells the extent of t-tubule penetration can vary substantially between cells and also between species (3). In a wide range of species (rat, guinea pig, cat, pig, human) atrial cell t-tubules are substantially less developed than in ventricular cells (4,5). In these cells the bulk of Ca signaling occurs on the cell boundary and penetrates to the interior via diffusion (6–8). However, these studies also find substantial cell-to-cell variability so that the presence of t-tubules in a population of cells can range between sparse and virtually absent. On the other hand, studies in the atria of large mammals (sheep, cow, horse) reveal that these cells display a moderately developed t-tubular structure with some penetration into the cell interior (4,9). In this case, Ca release occurred more similarly to ventricular cells, although large spatial gradients from the boundary to the interior were observed. In a recent study, Arora et al. (10) analyzed the distribution of t-tubule density in intact dog atrial cells. They found that the t-tubule distribution in these cells was mostly sparse, and substantially less developed than in ventricular cells. Also, they observed extensive cell-to-cell and regional variations in t-tubule density. In particular, they showed that almost 25% (12.5%) of atrial myocytes in the right (left) atrium did not display any t-tubule structure at all. These results indicate that the distribution of t-tubules in atrial cells can vary substantially between cells.

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*Correspondence: yshiferaw@csun.edu
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Ca release at an RyR cluster is typically initiated by a rise in Ca concentration due to a nearby LCC channel opening. However, under certain conditions, such as an elevated SR load, RyR clusters can fire in response to an increase in Ca concentration due to diffusion from a neighboring spark (6,11). In this case, Ca release can occur in a domino-like fashion leading to a wave front of Ca release in the cell. These excitations are referred to as "spontaneous Ca waves" because they are usually triggered by local fluctuations in Ca release among RyR clusters (12,13). These spontaneous Ca waves are believed to be highly arrhythmogenic because they can induce membrane depolarization due to inward NaCa exchange current (14–16). In atrial cells it is believed that spontaneous Ca release induces ectopic activity, which is responsible for initiation and maintenance of atrial fibrillation (AF) (17–19). Also, Ca cycling instabilities can induce dynamical heterogeneities in atrial tissue that may serve as a substrate for AF. Thus, the effect of Ca on AF is multifactorial, and likely to contribute as both the trigger and substrate for AF maintenance. However, up to now, the spatiotemporal dynamics of subcellular Ca in paced atrial cells has still not been fully understood. In particular, it is not known what kind of pacing-induced instabilities can occur in these cells.

In an elegant study, Thul et al. (6) constructed a simplified reaction diffusion model of Ca dynamics in atrial myocytes. This model accounted for the unique architecture of the atrial cell and predicted rich spatiotemporal dynamics. In particular, they identified the presence of ping waves in which Ca release at the cell boundary propagates to the cell interior via rotating waves along Z planes (6,20). They argued further that the interplay between boundary-induced waves and normal Ca release would destabilize Ca cycling and could therefore be proarrhythmic (21). These studies are important because, to our knowledge, they are the first to suggest that the spatial geometry of Ca release sites can be crucial to instabilities that may lead to cardiac arrhythmias.

In this article, we explore this direction further by applying a physiologically detailed computational model of Ca cycling (22) in atrial myocytes. This model accounts for experimentally based Markovian dynamics of RyR and LCC channels, along with Ca fluxes due to a range of Ca cycling proteins. Our main finding is that under rapid pacing with an action potential (AP) clamp, atrial myocytes exhibit Ca waves that are nucleated at the cell boundary and proceed to spread throughout the cell interior. These Ca waves are referred to as "triggered waves" because they are only nucleated during the AP when a critical number of Ca sparks are ignited by LCC channel openings at the cell boundary. This feature of triggered waves makes them fundamentally different from spontaneous Ca waves, which are triggered by stochastic fluctuations of RyR that are independent of LCC channel openings. Consequently, the latency to triggered waves is substantially shorter than for spontaneous waves, which only occur after a long pause. Hence, triggered waves can be highly arrhythmogenic because they occur with short latency and can induce triggered activity during the AP. We analyze triggered waves in detail and show that their onset displays a sharp sigmoid dependence on the SR load. Furthermore, we show that this nonlinearity explains our observation of aperiodic Ca cycling in response to a periodic AP waveform. By visualizing subcellular Ca during pacing we show that this Ca cycling abnormality is caused by the complex interaction between paced Ca release at the cell boundary and triggered excitations that can propagate into the bulk of the cell. We argue that this nonlinear instability is unique to atrial cells, and can serve as a potential mechanism for the induction and maintenance of AF.

METHODS

Computational cell model

Spatially distributed cell model. To model the spatiotemporal distribution of Ca in atrial myocytes, we have implemented an established mathematical model due to Restrepo et al. (22) and Restrepo and Karma (23) (i.e., the Restrepo model). In this model, the cell interior is divided into an array of compartments that represent distinct intracellular spaces. The Ca concentration within these compartments is treated as spatially uniform, and neighboring compartments are diffusively coupled. To model the atrial cell architecture, we first distinguish compartments that are close to the cell membrane, where LCC and RyR channels occupy the same dyadic junction, and compartments away from the cell membrane that do not sense Ca entry due to LCCs. For convenience, we will refer to compartments near the membrane as junctional Ca release units (CRUs), and all other compartments as nonjunctional CRUs. To model each compartment, we denote the Ca concentration in compartment α as c_α^n, where the superscript n indicates the location of that compartment in a 3D grid representation of the cell interior. In this study we will label our units according to the scheme $n = (n_x,n_y,n_z)$, where n_x denotes the longitudinal direction, n_y is the width of the cell, and n_z is the height.

In Fig. 1, *A* and *B*, we show an illustration of the various compartments that comprise a junctional (nonjunctional) CRU near the cell membrane. The intracellular compartments described in the model are: 1) the proximal space with concentration c_p^n and volume v_p. This compartment represents the volume of the cell that is in the immediate vicinity of the local RyR cluster. For junctional CRUs, this space includes 1–5 LCC channels along with a cluster of 100 RyR channels, whereas for nonjunctional CRUs, there are no LCC channels in the compartment. For junctional CRUs we will follow the Restrepo model, and take v_p to be the volume between the JSR and the cell membrane, which is roughly a pillbox of height 10 nm and diameter 100 nm. For nonjunctional CRUs, we note that recent experiments in atrial myocytes indicate the presence of large axial tubule structures that are closely associated with RyR clusters, and which likely restrict the local diffusion of Ca (24). However, the precise spatial arrangement of these structures at the scale of the RyR cluster are still not completely known. Thus, in this study we will first consider the case where the proximal space volume v_p of nonjunctional sites is the same as that of junctional sites. After this analysis, we will then analyze how our main results change when the volume v_p of nonjunctional sites is increased. 2) The submembrane space, with concentration c_s^n and volume v_s, which represents a volume of space in the vicinity of the proximal space, but smaller than the local bulk myoplasm. For junctional CRUs, we follow the Restrepo model and take v_s to be 5% of the cytosolic volume within a CRU. This volume includes sodium-calcium exchange channels (NCX) that are regulated by Ca concentrations that vary much more quickly than the average Ca concentration in the myoplasm. For nonjunctional CRUs we will again consider a range of