Ca^{2+} leak—What is it? Why should we care? Can it be managed?

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For arrhythmia triggers that are secondary to dysfunctional intracellular Ca^{2+} cycling, there are few, if any, agents that specifically target the Ca^{2+} handling machinery. However, several candidates have been proposed in the literature. Here we review the idea that these agents or their derivatives will prove invaluable in clinical applications in the future.

KEYWORDS Arrhythmia; Calcium; Delayed afterdepolarization; Pharmacology; Trigger

(Heart Rhythm 2017;■:1–8)

Introduction

Under normal conditions for all cardiac cells, during systole, Ca^{2+} influx through the cardiac calcium channel provides a trigger for calcium release from the sarcoplasmic reticulum (SR) through a large SR membrane, ligand-operated, ion channel called the ryanodine receptor (RyR). The open probability of the RyR protein is increased by elevation of cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$. Thus, Ca^{2+} entry into the cell produces a small increase of Ca^{2+}, which leads to opening of the RyR and subsequent release of a larger amount of Ca^{2+} that is stored in the SR. This process is known as calcium-induced calcium release (CICR) (Figure 1). Microscopic signals resulting from clusters of RyR openings generate Ca^{2+} signals called Ca^{2+} sparks. Spatial and temporal summation of action potential–evoked Ca^{2+} sparks underlies the global Ca^{2+} transient, which in contractile cells has a familiar rise and decay as Ca^{2+} is released and retaken up into the SR ready for the next heartbeat. Any remaining cytosolic Ca^{2+} is pumped out of the cell by sodium/calcium exchanger protein (NCX). Under normal conditions, CICR that occurs does not propagate but rather remains controlled by L type Ca^{2+} channel influx.

So what is "Ca^{2+} leak" if the cell always has spontaneous Ca^{2+} sparks, albeit at low probability?

When a cell is "overloaded" with calcium, the associated sequestration of Ca^{2+} by the SR can increase SR Ca^{2+} content to above normal levels. Under these circumstances, the Ca^{2+} leaks out of the SR in the form of Ca^{2+} waves. These are local Ca^{2+} release events that trigger regenerative Ca^{2+} waves via the CICR process. The Ca^{2+} wave can propagate throughout the cell and in some cases can trigger Ca^{2+} waves in an adjacent cell (Figure 2).[1] It seems that intracellular Ca^{2+} waves generally occur when the SR Ca^{2+} content is elevated above a threshold value,[2,3] but other changes, such as altered Ca^{2+} sensitivity of the RyR, can induce Ca^{2+} waves. Some of the Ca^{2+} in the wave is pumped out of the cell by the electrogenic NCX. The resulting current depolarizes the membrane (producing a delayed afterdepolarization [DAD] like membrane voltage change) and can be sufficient to initiate an action potential. Yet synchrony of Ca^{2+} releases between coupled cells is required to provide sufficient depolarizing current within 1 region to initiate an arrhythmic action potential in an intact ventricle/atrium. The critical number of coupled cells experiencing a DAD is a topic of debate and research.[4–6]

SR Ca^{2+} leak is increased in numerous pathological conditions, such as heart failure (HF)[7] and postmyocardial infarction.[8,9] SR Ca^{2+} leak, if persistent, decreases SR Ca^{2+} load and, as explained earlier, can lead to propagating Ca^{2+} waves and thus DADs (Figure 3).

Although Ca^{2+} leak is an operational term, several mechanisms have been proposed to explain the altered RyR gating that leads to Ca^{2+} leak. An increased sensitivity of RyR to its ligand cytosolic Ca^{2+} may be due to enhanced protein kinase A (PKA) and/or calmodulin-dependent protein kinase II (CaMKII)-dependent RyR[7] phosphorylation at specific sites.[10–13] Recent data using human tissues favor one idea in which Ca^{2+} handling abnormalities in HF are due to excessive CaMKII phosphorylation at a specific RyR residue.[14,15] Other factors such as the oxidative state could

Dr. Boyden is supported by National Institutes of Health Grant HL114383. Dr. Smith has interests in Clyde Biosciences Ltd UK. **Address reprint requests and correspondence:** Dr. Penelope A. Boyden, Department of Pharmacology, Columbia College of Physicians and Surgeons, 630 West 168th St, New York NY 10032. E-mail address: pab4@columbia.edu.

1547-5271/$-see front matter https://doi.org/10.1016/j.hrthm.2017.11.018

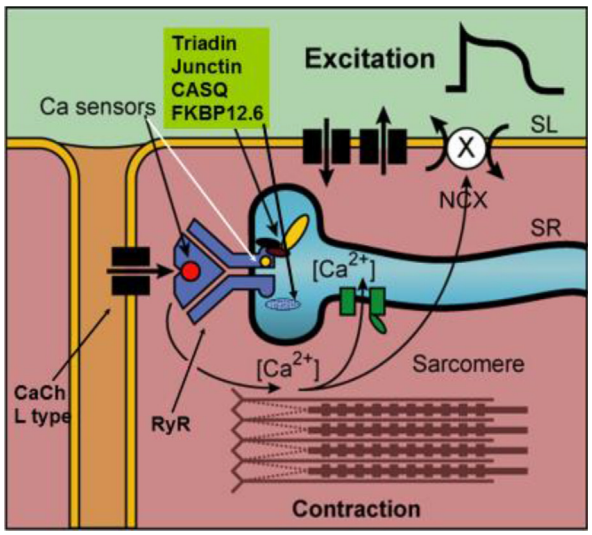

Figure 1 Simple diagram of the excitation–contraction coupling system in the cardiac cell. During the action potential, Ca^{2+} enters the cell as a rapid influx, followed by a maintained component of the slow inward Ca^{2+} current (thick arrow). The rapid influx of Ca^{2+} via the t- tubules is thought to induce release of Ca^{2+} from a release compartment in the sarcoplasmic reticulum (SR) by triggering opening of Ca^{2+} channels via binding sites (sensors) on ryanodine (RyR) protein. Relaxation follows when the cytosolic Ca^{2+} is sequestered again in an uptake compartment of the SR (SERCA pump; *green boxes*) and partly extruded through the cell membrane by the Na^{+}/Ca^{2+} exchanger (NCX). The process of NCX is electrogenic so that Ca^{2+} extrusion through the NCX leads to a depolarizing current. (From Ter Keurs HEDJ, Boyden PA. Calcium and arrhythmogenesis. Physiol Rev 2007;87:457–506.[75])

change, resulting in direct activation of the RyR protein.[16] Finally, others have suggested that RyR gating may be altered when an abundance of endogenous proteins that modulate RyR are altered (e.g., sorcin, S100A[17,18]).

Mutations and dysregulation of RyR and other calcium binding proteins have been implicated in several gene-based arrhythmias, such as RyR and catecholaminergic polymorphic ventricular tachycardia (CPVT), and calsequestrin and CPVT.[19] The mechanisms of these arrhythmias are similar to those of acquired diseases, that is, the arrhythmic events are caused by abnormally propagating Ca^{2+} waves, which cause NCX-dependent membrane oscillations (DADs) and triggered beats (Figures 2 and 3).

Can Ca^{2+} leak be managed?

As an antiarrhythmic drug, we would want the agent to modulate the mishandled Ca^{2+} so that Ca^{2+} does not increase Ca^{2+}-dependent currents to cause depolarization and elicit action potentials. If we target the spontaneous Ca^{2+} releases, then we would reduce the initiators of the Ca^{2+} waves—the DADs—thus triggering beats.

The arrhythmias mentioned result when the cell's SR Ca^{2+} content is increased above a threshold level at which waves are produced. Recent work suggests that a decrease of threshold (due to sensitization to RyR Ca^{2+} release) also produces Ca^{2+} waves and DADs. For arrhythmias seen in HF, the involvement of DADs in some ventricular arrhythmias has been shown.[20,21] However, in HF the SR Ca^{2+} content is decreased, suggesting that the threshold for Ca^{2+} release may be lower, such that Ca^{2+} waves would occur at a lower SR Ca^{2+} content. This may be a consequence of increased leakiness of the RyR during diastole, such that there is increased Ca^{2+} efflux at a given SR Ca^{2+} content. The exact molecular mechanisms responsible for this are controversial,[22] but as above, it may be associated with increased phosphorylation of the RyR due to PKA or CaMKII.[14]

An example of the occurrence of DADs in the absence of increased SR Ca^{2+} content is provided by CPVT. This arrhythmia in patients occurs during exercise or other stress. The similarity of the abnormalities in the ECGs to those observed in patients with digitalis toxicity led to the suggestion of similarities in an underlying mechanism. Genetic studies have shown that many CPVT patients have a mutation in RyR (e.g., R4496C) or the intrasarcoplasmic protein calsequestrin (e.g., R33Q). The current hypothesis is that the mutated protein causes increased leak of Ca^{2+} from the SR, so that Ca^{2+} waves and DADs occur at a lower SR Ca^{2+} content than in controls (Figure 3).[23]

Therapies for DAD-related arrhythmias

For these Ca^{2+}-dependent (Ca^{2+} wave–dependent) arrhythmias, the goal of therapy is (1) to prevent the DAD from occurring and/or (2) to prevent the DAD from triggering an action potential.

The latter can potentially be achieved using sodium channel blockers. A better solution, however, would be to remove the underlying DAD directly. In the case of arrhythmias resulting from "calcium overload," it may be possible to remove the underlying "overload." For example, local

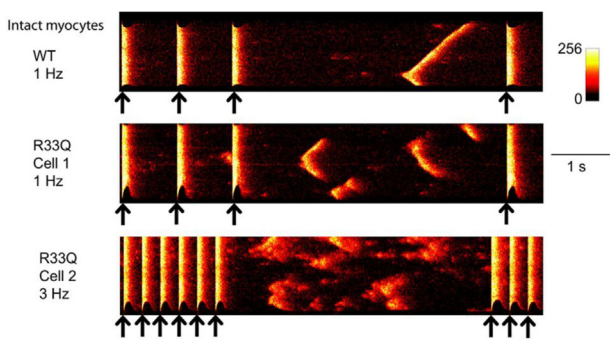

Figure 2 Representative confocal line scan images showing spontaneous Ca^{2+} release events (SCaEs) in wild-type (WT) and R33Q (catecholaminergic polymorphic ventricular tachycardia mutation in calsequestrin) cells in the presence of isoproterenol. *Black arrows* indicate field stimulations. SCaEs in WT myocytes were usually due to a cell-wide wave that was initiated at 1 site (*red arrow*). SCaEs in diseased R33Q cells varied. Often, fragmented spontaneous Ca^{2+} waves occurred and slowly propagated (cells 1 and 2), and wavelets and Ca^{2+} sparks occurred before Ca^{2+} transients resumed the diastolic level. (From Liu N, Denegri M, Dun W, et al. Abnormal propagation of calcium waves and ultrastructural remodeling in recessive catecholaminergic polymorphic ventricular tachycardia. Circ Res 2013;113:142–152.[76])