Published in final edited form as:

Pediatr Cardiol. 2012 August ; 33(6): 968–974. doi:10.1007/s00246-012-0236-5.

Imaging Arrhythmogenic Calcium Signaling in Intact Hearts

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Abstract

Protein complex of the cardiac junctional sarcoplasmic reticulum (SR) membrane formed by type 2 ryanodine receptor, junction, triadin, and calsequestrin is responsible for controlling SR calcium (Ca) release. Increased intracellular calcium (Ca_i) activates the electrogenic sodium–Ca exchanger current, which is known to be important in afterdepolarization and triggered activities (TAs). Using optical-mapping techniques, it is possible to simultaneously map membrane potential (V_m) and Ca_i transient in Langendorff-perfused rabbit ventricles to better define the mechanisms by which V_m and Ca_i interactions cause early afterdepolarizations (EADs). Phase 3 EAD is dependent on heterogeneously prolonged action potential duration (APD). Electrotonic currents that flow between a persistently depolarized region and its recovered neighbors underlies the mechanisms of phase 3 EADs and TAs. In contrast, “late phase-3 EAD” is induced by APD shortening, not APD prolongation. In failing ventricles, upregulation of apamin-sensitive Ca-activated potassium (K) channels (I_{KAS}) causes APD shortening after fibrillation-defibrillation

episodes. Shortened APD in the presence of large Ca_i transients generates late-phase 3 EADs and recurrent spontaneous ventricular fibrillation. The latter findings suggest that I_{KAS} may be a novel antiarrhythmic targets in patients with heart failure and electrical storms.

Keywords

Triggered activity; After depolarization; Ventricular fibrillation; Calcium dynamics; Optical mapping

Introduction

Protein complex of the cardiac junctional sarcoplasmic reticulum (SR) membrane formed by type 2 ryanodine receptor, junction, triadin, and calsequestrin is responsible for controlling SR calcium (Ca) release [25]. Increased intracellular Ca (Ca_i) activates the electrogenic sodium-Ca exchanger current (I_{NCX}), causing depolarization and triggered activities (TAs) [20]. Choi and Salama [7] pioneered methods to simultaneously map membrane potential (V_m) and Ca_i transient in Langendorff-perfused rabbit ventricles. We have adapted their methods to investigate the relationship between Ca_i transient and ventricular arrhythmogenesis.

Methods of Optical Mapping

All study protocols were approved by the Institutional Animal Care and Use Committees. Animals were anesthetized before any intervention. Most of our studies used New Zealand white rabbits. The hearts were removed while the rabbits were under general anesthesia and were immediately Langendorff-perfused with 37°C oxygenated Tyrode's solution equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.40 ± 0.05 . The coronary perfusion pressure was regulated and maintained at 70–80 cmH₂O. The heart was then stained with Rhod-2 Am for Ca_i and RH237 for V_m . The hearts were illuminated with a laser (Verdi; Coherent, Santa Clara, CA) at a wavelength of 532 nm. The fluorescence was filtered and acquired simultaneously with two complementary metal–oxide–semiconductor cameras (BrainVision, Tokyo, Japan) at 2 ms/frame and 100×100 pixels with spatial resolution of 0.35×0.35 mm²/pixel. We performed optical mapping of either epicardial or endocardial surface. The average fluorescence level ($\bar{F}$) of an individual pixel was first calculated for the duration of recording. The ratio on each pixel was then calculated as $(F - \bar{F})/\bar{F}$. The image data were spatiotemporally filtered with $3 \times 3 \times 3$ averaging. To generate the ratio maps, shades of red were assigned to represent greater-than-average fluorescence (depolarization), and shades of blue were assigned to represent lower-than-average fluorescence (repolarization).

Types of Afterdepolarizations

Afterdepolarizations can be classified into early afterdepolarization (EAD), delayed afterdepolarization (DAD), and late-phase 3 EAD [6]. All three forms of afterdepolarizations are initiated by spontaneous depolarizations during phase 2, 3, or 4 of the action potential. We will focus the discussion on the mechanisms of phase 3 EAD and late-phase 3 EAD, which are two completely different phenomena. Table 1 lists the two different types of afterdepolarizations. More expanded discussion is given later in the text.

Phase 3 EADs and TA

Previous studies have shown that EADs are strongly associated with ventricular arrhythmias in long QT syndrome (LQTS). The mechanisms of arrhythmogenesis are thought to be due

to either phase 2 or 3 EADs. It is generally accepted that phase 2 EADs result from the reactivation of L-type calcium current ($I_{Ca,L}$) and/or spontaneous Ca release from the SR [8, 12, 21]. The ionic mechanism of phase 3 EADs, however, is less clear. A review of literature showed that phase 3 EADs have usually been reported in intact tissue preparations, such as Purkinje fibers or ventricular muscle [2, 10, 11, 17, 19]. It is rarely observed in single cells. It is possible that most phase 3 EADs observed in tissues are not a genuine cellular-level phenomenon but instead are a consequence of “prolonged repolarization-dependent re-excitation” [3]. The latter phenomenon occurs because the dispersion of repolarization is enhanced in LQTS. A voltage gradient between long and short APD regions could create a “boundary” current that electrotonically depolarizes the short APD region as it tries to repolarize, generating TA. Maruyama et al. [14] tested this hypothesis by performing high-resolution optical mapping of Ca_i and membrane voltage (V_m) in a rabbit model of acquired LQTS, which was accompanied by computer simulations that reproduced the experimental observations. The experiments were performed in Langendorff-perfused normal rabbit hearts using simultaneous Ca_i - and V_m -mapping techniques. EADs and TAs were induced by bradycardia after endocardial cryoablation, a selective rapid delayed rectifier potassium current (I_{Kr}) blocker E-4031 ($0.5 \mu\text{mol L}^{-1}$; Tocris Bioscience, Ellisville, MO), and 50% decrease of extracellular potassium ($[K^+]_0$) and magnesium ($[Mg^{2+}]_0$) concentrations [8].

Exposure to $0.5 \mu\text{mol L}^{-1}$ E-4031 in combination with a 50% decrease in $[K^+]_0$ and $[Mg^{2+}]_0$ prolonged QT intervals and induced R-on-T ectopic beats in all hearts studied. Because the QT interval was prolonged, both APD dispersion and maximal voltage gradient (VG) during repolarization increased. The voltage gradient occurred because APD prolongation was spatially heterogeneous, causing “island”-like long APD regions to emerge (Fig. 1a), with a large VG at the boundary zone between the long and short APD regions. Phase 2 EADs and R-on-T ventricular ectopic beats were observed after decreasing $[K^+]_0$ and $[Mg^{2+}]_0$. Phase 2 EADs further enhanced heterogeneity of repolarization because they occurred exclusively in long APD regions. Phase 3 EADs became manifest after E-4031 infusion with low $[K^+]_0$ and $[Mg^{2+}]_0$ (Fig. 1b). BAPTA-AM, a Ca_i chelating agent, decreased the maximal amplitude of Ca_i transient and abolished phase 2 EADs, but phase 3 EADs persisted after BAPTA-AM loading in all hearts studied. These findings suggest that whereas phase 2 EADs are Ca_i -dependent, phase 3 EADs are not. Interestingly, the largest phase 3 EADs always occurred at the boundary between long and short APD regions. The EAD amplitude correlated with VG at the time of EAD onset at the site with the largest phase 3 EAD. Taken together, the findings suggest that electrotonic currents flowing from more positive V_m in long APD regions to shorter APD regions can cause phase 3 EADs at the boundary zone without there being any requirement for SR Ca release.

Late Phase 3 EADs and Their Role in the Initiation of Cardiac Fibrillation

As listed in Table 1, “late-phase 3 EAD” and “phase 3 EAD” are different mechanisms of arrhythmogenesis. The term “late-phase 3 EAD” was coined by Burashnikov and Antzelevitch [4]. This mechanism of arrhythmogenesis may be responsible for immediate recurrence of atrial fibrillation (AF) after cardioversion, and may also be relevant to other types of cardiac arrhythmias [16, 18]. While trying to study the mechanisms of ventricular fibrillation (VF) in rabbits with pacing-induced heart failure, Ogawa et al. discovered that recurrent VF (electrical storm) occurred in some of the failing ventricles. Figure 2a shows a continuous pseudo-electrocardiogram (pECG) recording in a failing heart. The initial sinus rhythm was interrupted by pacing-induced VF, which was terminated by defibrillation shocks (green arrows) and followed by seven spontaneous VF (SVF) and one spontaneous ventricular tachycardia episodes in rapid succession. This sequence of events simulates the electrical storm seen in human patients. Figure 2b, c, d show the V_m recording (white line) and the Ca_i (yellow line) optical signals obtained during baseline sinus rhythm immediately

after direct current termination of pacing-induced VF and spontaneous conversion of sinus rhythm to SVF4, respectively. There was spontaneous Ca_i increase in a second post-shock beat (shown in Fig. 2c; (yellow arrow)). Although the Ca_i increase was associated with DAD, no VF was initiated. Afterward, the APD is abbreviated, but the Ca_i is large. Persistent increase of Ca_i beyond the end of the action potential created a late-phase 3 EAD (green dot). Figure 2d shows SVF4. The first beat of SVF occurred during late phase 3 of a short AP and when Ca_i was still increased, which is consistent with late-phase 3 EAD. Figure 2e shows single beats at four different time points during the experiment, including baseline, after fibrillation–defibrillation (Eb), immediately before SVF4 (Ec), and 15 min after SVF7. Figure 2f shows time-dependent changes of APD and intracellular calcium transient duration (Ca_iTD) after defibrillation, showing the transient nature of the APD shortening. Late-phase 3 EAD-induced after defibrillation, showing the transient nature of the APD shortening. Late-phase 3 EAD-induced triggered extrasystoles represent a new concept of arrhythmogenesis in which abbreviated repolarization permits *normal SR Ca release* to induce an EAD-mediated closely coupled triggered response, particularly under conditions permitting Ca_i loading. These EADs are distinguished by the fact that they interrupt the final phase of repolarization of the action potential (late phase 3). Compared with previously described DAD or Ca_i -dependent EAD, it is *normal Ca-induced Ca release*, not spontaneous SR Ca release, that is responsible for the generation of the EAD.

Small-Conductance Ca-Activated Potassium Channel and Late-Phase 3 EAD

Late-phase 3 EAD occurs only in failing hearts and is associated with APD shortening. The mechanisms by which VF-defibrillation episodes are followed by APD shortening remain unclear. Because VF is associated with Ca_i accumulation, especially in failing ventricles [1, 13, 24], we hypothesized that apamin-sensitive small-conductance Ca^{2+} -activated K^+ (SK) channels may be in part responsible for post-shock APD shortening. Apamin is a neurotoxin that selectively blocks SK channels [5, 26]. Previous studies in cardiac tissues showed that apamin-sensitive K^+ current (I_{KAS}) is abundantly present in cardiac atrial cells but not in normal ventricular cells [15, 23]. We were not able to find any studies on I_{KAS} regulation in failing ventricles. Therefore, we [9] studied normal and failing rabbit hearts to determine if I_{KAS} is upregulated in failing ventricles. A rabbit model of tachycardia-induced heart failure was used. Simultaneous optical mapping of Ca_i and V_m was performed in Langendorff-perfused failing hearts and normal hearts. The results show that after fibrillation–defibrillation episodes, acute but transient shortening of APD occurred in all failing ventricles. Figure 3a (left panel) shows V_m (black line) and Ca_i (red line) optical signals recorded during one of the SVF episodes. There were 2 post-shock beats (beats 1 and 2) before VF termination. Optical maps confirmed complete cessation of wave fronts after beat 2, consistent with type B successful defibrillation [22]. However, there was a large Ca_i transient and short APD (beat 3). The first beat of SVF (beat 4) began during late phase 3 of the preceding sinus beat. In Figure 3a (b), an isochronal map illustrates that beat 4 originated from the basal portion of the right ventricle. The right two columns are ratio maps of V_m and Ca_i , respectively, of the same beat. Ca_i remained increased throughout the mapped field, whereas V_m had already repolarized. The focal beat originated from the left upper quadrant during persistent Ca_i increase, consistent with the late-phase 3 EAD mechanism [4, 15]. Five SVF episodes were recorded after initial successful defibrillation in this failing heart. Apamin prevented both the post-shock APD shortening and SVF (Fig. 3b). We repeated fibrillation–defibrillation episodes four times, but no SVF was observed during the post-shock period after apamin.

To directly document the upregulation of I_{KAS} in failing ventricles, we performed patch clamp studies in failing and normal ventricles. The density and properties of I_{KAS} were

examined in cardiomyocytes isolated from normal and failing rabbit left ventricles using the voltage-clamp technique in whole-cell mode. Figure 4a shows representative current traces obtained with a step-pulse protocol in the absence and presence of 100 nM apamin in the bath solution. Mean I_{KAS} density (determined as the apamin-sensitive difference current) was significantly greater in failing than in normal ventricular epicardial myocytes. In contrast, when the intrapipette free- Ca^{2+} was buffered to 100 nM, the apamin-sensitive current was much smaller, with no significant difference between normal and failing ventricular myocytes (Fig. 4d). Figure 4b illustrates the I_{KAS} -voltage (I-V) relationships. Transmural distribution of I_{KAS} in failing ventricles was studied using cardio-myocytes isolated from three layers (six epicardial cells, five midmyocardial cells, and seven endocardial cells from five animals). Mean I_{KAS} density in epicardial myocytes was significantly greater than midmyocardial cells and endocardial cells (Fig. 4c). To further elucidate the mechanisms underlying I_{KAS} upregulation, Ca^{2+} -dependence of I_{KAS} was studied in epicardial cells using pipette solutions containing increasing intracellular free Ca^{2+} concentrations. Figure 4d demonstrates that the steady-state Ca^{2+} response of I_{KAS} was leftward-shifted in failing cells compared with the normal cells. The data were fitted with the Hill equation and yielded K_d of 232 ± 5 nM for failing and 553 ± 78 nM ($p = 0.002$) for normal cells and Hill coefficients of 2.38 ± 0.13 for failing and 1.50 ± 0.30 for normal cells ($p = 0.01$). These results are compatible with the notion that the K^+ channels carrying I_{KAS} have increased sensitivity to cytosolic Ca^{2+} in failing ventricles.

Summary

In summary, simultaneous optical mapping of Ca_i and V_m demonstrated new mechanisms of EADs and cardiac arrhythmogenesis. Phase 3 EAD, which is dependent on prolonged APD, occurs through electrotonic interaction between regions with long APD and short APD. The large VG caused electrotonic current, which results in EAD in phase 3, TA, and arrhythmia. In contrast, late-phase 3 EAD is associated with APD shortening and persistent Ca_i increase. In failing ventricles, upregulation of I_{KAS} underlies the mechanisms of post-shock APD shortening and recurrent SVF. The latter findings suggest that I_{KAS} may be a novel antiarrhythmic targets in patients with heart failure.

Acknowledgments

This study was supported in part by National Institutes of Health Grants no. P01 HL78931, R01 HL78932, and HL71140 as well as a Medtronic-Zipes endowment.

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Fig 1.

Development of phase 2 and 3 EADs. **a** The spatial distribution of APDs and voltage gradients (VG) during repolarization is displayed in color-scaled maps. Optical APs at the maximal (*filled squares*) and minimal (*unfilled squares*) APD sites are shown with pECG. In addition to E-4031, decreasing $[K^+]_o$ and $[Mg^{2+}]_o$ greatly enhanced repolarization heterogeneity. Note that the appearance of the phase 2 EAD (*filled circle*) further increased the VGs and was associated with emergence of an R-on-T ventricular ectopic beat (*asterisk*). APD was not measurable in a blank area of the APD map where repolarization was interrupted by the ectopic beat. **b** With E-4031 alone, there was no phase 3 EAD. Phase 3 EAD (*unfilled circles*) and ectopic beats (*asterisk*) emerged after a decrease in $[K^+]_o$ and $[Mg^{2+}]_o$. Phase 3 EAD is discernable as the V_m difference between the resting V_m and the first deviation from the smooth contour during phase 3 repolarization. Reproduced from Maruyama et al. [13] with permission

Fig 2.

VF storm in a failing heart. There were a total of seven episodes of SVF within 20 min after initial successful defibrillation in this rabbit heart. **a** Continuous recording of pECG. **b** Baseline V_m (white line) and Ca_i (yellow line) recordings. **c**, **d** V_m and Ca_i at termination and at onset of SVF, respectively. Yellow arrow points to post-shock spontaneous Ca increase, which was associated with DAD. Afterward, there was an abbreviated action potential and late-phase 3 EAD (green dot). The large persistent Ca_i increase may be responsible for the occurrence of the late-phase 3 EAD. Note the presence of short APD in the immediate post-shock period (c) and that the first ectopic beat initiating VF occurred from late phase 3 of the preceding action potential (d). The tracings in red boxes in **b** through **d** are also shown in (e), which highlights the V_m and Ca_i changes at different time points during the study. There was transient shortening of APD and, to the lesser extent, Ca_iTD after defibrillation. **f** Measurements of APD and Ca_iTD , depicting the transient nature of these changes. The time points “b” and “c” are marked as Eb and Ec, respectively, in **a**. The time points “a” and “d” are from baseline and 31 min after the last episode of SVF, respectively. Time point “d” is outside of the range and is not part of the figure. Modified from Ogawa et al. [15] with permission

Fig 3.

Pretreatment with apamin prevents late-phase 3 EAD and refrillation. The *black* and *red lines* indicate optical tracings for V_m and Ca_i , respectively, obtained from the endocardial surface in a failing ventricle. **A (a)** Before apamin, a defibrillation shock (*arrow*) was followed by two post-shock beats (1 and 2). Optical maps (not shown) confirmed complete cessation of wave fronts after beat 2, consistent with type-B successful defibrillation. After a short pause, there was a large Ca_i transient (*red dot*), short APD (beat 3), and first beat (beat 4) of SVF arising from late phase 3. **A (b)** Snapshots of Ca_i and V_m ratio maps at times from 10 ms before to 20 ms after the onset of beat 4. Note that Ca_i remained increased throughout the mapped field, whereas V_m has already repolarized. Beat 4 increased during persistently high Ca_i and initiated the SVF. **b** After apamin ($1 \mu\text{M}$) infusion, post-shock beats 5 and 6 had longer APD than beats 1 through 4 in (**A**). APD and $Ca_i\text{TD}$ were approximately the same, and SVF episodes were completely prevented. *PM* papillary muscle, *S* interventricular septum. From Chua et al. [9] with permission

Fig 4.

I_{KAS} in failing rabbit ventricles. **a** Representative K^+ current traces obtained from normal (*upper panel*) and failing (*lower panel*) ventricular myocytes. Voltage-pulse protocol is shown in the inset. *Baseline* shows current traces in the absence of apamin ($I_{baseline}$). “Apamin 100 nM” shows currents in the presence of 100 nM apamin (I_{apamin}). “Difference” shows I_{KAS} calculated as $I_{baseline} - I_{apamin}$. **b** $I-V$ relationship obtained from normal ($N = 6$) and failing ($N = 6$) ventricular epicardial cells. **c** I_{KAS} density at 0 mV with an intrapipette free- Ca^{2+} of 863 nM recorded from epicardial (Epi), midcardial (Mid), and endocardial (Endo) cells from five failing ventricles. $*p < 0.05$. **d** Steady-state Ca^{2+} response of I_{KAS} in normal and failing epicardial ventricular myocytes. The data were fitted with the Hill equation: $y = 1/[1 + (K_d/X)^n]$, where y represents I_{KAS} normalized to the currents with 10 μM intrapipette Ca^{2+} ; X is the concentration of Ca^{2+} ; K_d is the concentration at half-maximal activation; and n is the Hill coefficient. Error bars represent SEM. Numbers in parentheses indicate the number of cells patched. From Chua et al. [9] with permission

Table 1

Phase 3 EAD versus Late-phase 3 EAD

Phase 3 EAD	Late-phase 3 EAD
Associated with APD prolongation	Associated with APD shortening
Mostly induced by electrotonic current across steep APD gradient	Induced by I_{NCX} activation due to persistent Ca^{2+} increase in late phase 3
May underlie the mechanisms of (1) drug-induced long QT and sudden death and (2) increased arrhythmia during heart failure and hypokalemia	May underlie the mechanisms of (1) immediate recurrence of AF after cardioversion and (2) electrical storm in patients with heart failure