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Delayed Afterdepolarization in Intact Canine Sinoatrial Node as a Novel Mechanism for Atrial Arrhythmia

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Abstract

Introduction—Recent evidence indicates that spontaneous sarcoplasmic reticulum Ca release and Na-Ca exchanger current activation contribute to the sinoatrial node (SAN) automaticity. These findings suggest that SAN activity may share mechanisms that underlie both automaticity and triggered activity. The aim of this study is to test the hypothesis that spontaneous, non-voltage gated, intracellular Ca (Ca_i) elevation may induce delayed afterdepolarization (DAD) in intact SAN during isoproterenol infusion.

Methods and Results—We simultaneously mapped Ca_i and membrane potential in 31 isolated Langendorff-perfused canine right atriums (RA). Isoproterenol increased heart rate and late diastolic Ca_i elevation (LDCAE) of the superior SAN, leading to consistent SAN automaticity in all 31 RAs. However, DAD-like diastolic depolarizations (DD) were transiently observed in 4 RAs during isoproterenol infusion. These DAD-like DDs were preceded by LDCAE, but did not trigger a full action potential. The LDCAE preceding DAD-like DDs had smaller amplitude (0.41 ± 0.08 AU vs. 0.48 ± 0.07 AU, p=0.001) and less steep slopes (3.7 ± 1.3 AU/s vs. 4.8 ± 1.4 AU/s, p=0.001) than that of sinus beats. The coupling interval of DAD-like DDs was longer than that of the preceding normal beats (407 ± 48 ms vs. 371 ± 44 ms, p=0.002).

Conclusion—The isoproterenol-induced LDCAE of superior SAN induced a full action potential in most cases. However, if the LDCAE was too small to trigger an action potential, then it induces only DAD-like DD. The failure of DAD-like DD to consistently trigger a sinus beat is a novel mechanism of atrial arrhythmogenesis.

Keywords

calcium dynamics; sympathetic nervous system; triggered activity; sinoatrial node; afterdepolarization

Introduction

Automaticity and triggered activity are thought to be two distinct mechanisms for the initiation of heart beats. Automaticity occurs spontaneously and can be a source of both normal and abnormal heart beats, while triggered activity is pacing-induced and is almost always pathological. A mechanism of triggered activity is spontaneous (non-voltage gated)

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sarcoplasmic reticulum (SR) Ca release, which causes Na-Ca exchanger current (I_{NCX}) activation and membrane depolarization, resulting in delayed afterdepolarization (DAD).[1] When DAD reaches threshold, it initiates triggered activity and arrhythmia (reverse excitation-contraction coupling).[2–3] Recent studies, however, showed that rhythmic spontaneous Ca release ("Ca clock") [4–6] may work together with hyperpolarization-activated membrane currents ("membrane clock") to generate normal sinus rhythm, a prototypical example of normal automaticity. These findings suggest that sinoatrial node (SAN) activity may share mechanisms that underlie both automaticity and triggered activity, i.e., I_{NCX} activation.[1, 7–13] Consistent with this hypothesis, Bogdanov et al [12] showed that in single isolated SAN cells, spontaneous SR Ca release in conditions of impaired I_{NCX} may result in membrane potential (V_m) oscillation without leading to regenerative action potential. Maltsev and Lakatta [5] used computer models to study the mutual dependence of the Ca clock and the membrane clock. The authors demonstrated that when membrane current (such as L-type Ca current, $I_{Ca,L}$) is inhibited, the SAN may demonstrate DADs rather than an action potential. Similarly, intracellular calcium (Ca_i) waves [14] may trigger V_m oscillations in ventricular tissues of intact hearts during isoproterenol infusion, suggesting that insufficient amount of SR Ca release may cause DADs but not full action potential in intact tissues. However, whether or not DADs can occur in the intact SAN remains unknown. It is also unclear if the DADs in the SAN can lead to atrial arrhythmia. The purpose of the present study was to test the hypothesis that the spontaneous intracellular Ca (Ca_i) elevation may induce DAD-like activity in intact SAN during isoproterenol infusion, and that failure of the DAD-like activity to trigger sinus beats is a mechanism of atrial arrhythmia.

Methods

Dual V_m and Ca_i recordings in Langendorff-perfused Canine right atrium preparations

This study protocol was approved by the Institutional Animal Care and Use Committee and conforms to the guidelines of the American Heart Association. We studied isolated canine right atriums (RA) in 31 mongrel dogs (22 to 28 kg). Among them, 11 were from a previous study,[15] 20 were from subsequently (unpublished) studies. The dogs were intubated and anesthetized with isoflurane. The chest was opened via median sternotomy and the heart was rapidly excised. The dogs were euthanized by exsanguination during general anesthesia. The right coronary artery was perfused with 37°C Tyrode's solution equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. The composition of Tyrode's solution was (in mmol/L): 125 NaCl, 4.5 KCl, 0.25 $MgCl_2$, 24 $NaHCO_3$, 1.8 NaH_2PO_4, 1.8 $CaCl_2$, and 5.5 glucose). The perfusion pressure was between 50 mmHg and 60 mmHg. All ventricular coronary branches were tied off. Both ventricles and the left atrium were excised and removed from the preparation. During optical recordings, contractility was inhibited by 10–17 μmol/L of blebbistatin.[15]

Dual V_m and Ca_i recordings

We used 0.5 mg of Rhod-2 AM (Molecular Probes) dissolved in 1 mL of dimethylsulfoxide containing Pluronic F-127 (20%wt/vol) to stain Ca_i. This solution was diluted in 300 ml of Tyrode's solution to achieve a final Rhod-2 concentration of 1.48 μmol/L and was infused into the heart over a 10-minute period. The heart was perfused with dye-free Tyrode's solution for 15–30 minutes for de-esterification of Rhod-2 AM. The hearts were then stained again by direct injection of voltage sensitive dye (RH237, Molecular Probes) into the perfusion system. The double-stained hearts were excited with laser light at 532 nm.[15] Fluorescence was collected using 2 cameras (MiCAM Ultima, BrainVision, Tokyo, Japan) at 1 ms/frame and 100 X 100 pixels with spatial resolution of 0.35 × 0.35 mm^2/pixel. The fluorescence was obtained through a common lens, separated with a dichroic mirror (650 nm

cutoff wavelength), and directed to the respective camera with additional filtering (715 nm long pass for V_m and 580 ± 20 nm for Ca_i). The sources of optical signals came from both the surface and up to 1–2 mm below the surface.[16] After dual V_m and Ca_i optical mapping of baseline spontaneous beats, isoproterenol was infused starting from 0.01 μmol/L and gradually increased to 1.0 μmol/L.

Data Analysis

Isoproterenol infusion increased heart rate and induced the Ca_i elevation at superior SAN during late diastolic period (arrow in Figure 1A).[15] This Ca_i elevation was defined as the late diastolic Ca_i elevation (LDCAE). The LDCAE induced diastolic depolarization (DD) and generated a sinus beat. However, when an LDCAE induced DD but failed to generate a sinus beat, the down slope of the DD became visible and showed same morphology as the DAD in other sites of heart. Therefore, we defined these DDs as the "subthreshold DADs" or "DAD-like activities".

The Ca_i and V_m traces were normalized to their respective peak-to-peak amplitude for comparison of timing and morphology. Considering the total amplitude of Ca_i and V_m tracings as 1 arbitrary unit (AU), the maximum amplitudes of LDCAE (b of Figure 2) and DD (d of Figure 2) were measured from baseline to the peak level of LDCAE and DD, respectively. The slopes of LDCAE (a of Figure 2) and DD (c of Figure 2) were measured from the onsets of LDCAE or DD to peak levels of LDCAE or DD, respectively. The onsets of LDCAE and DD were defined by the time of the transition between negative to positive values in dCa_i/dt and dV_m/dt curves.[15] We generated activation isochronal maps using the onset of phase 0 of the action potential in the leading pacemaker site as time 0. The time of the isochronal map is time when the V_m reached 50% of the maximum amplitude of phase 0. We compared the slopes and maximum amplitude of LDCAE and DD between subthreshold DADs (n=30) and adjacent sinus beats (n=32) with Student's t tests. Data were presented as mean ± SD. A p value of < 0.05 was considered significant.

Results

At baseline, the LDCAE was observed at the inferior SAN in only 4 (13%) RA preparations. However, a robust LDCAE occurred at the leading pacemaker site of superior SAN in all RA preparations during isoproterenol infusion.[15] Among 31 preparations studied, 37 episodes of subthreshold DADs were observed in 4 RA preparations during increasing doses of Isoproterenol. The following analyses are limited to the data from these 4 RAs. The remaining 27 RAs are not included in the statistical analyses.

Intermittent Subthreshold DAD

Three RAs had a total of 20 episodes of subthreshold DADs that occurred intermittently. Figure 1 shows optical maps of intermittent subthreshold DADs during 0.03 μmol/L isoproterenol infusion. The first beat (1) shows LDCAE (arrows in Ca_i tracings of Figure 1A) followed by the initiation of sinus beats from the same sites (sites b and c of Figure 1B(1) and Movie I), compatible with LDCAE-induced SAN activation. In comparison, LDCAE (asterisks) before the second beat (Figure 1A(2)) was associated with gradual rise and fall of the V_m but not a full action potential. These V_m changes (asterisks) were consistent with subthreshold DAD induced by LDCAE. A latent pacemaker in the inferior RA then generated the second beat, as shown by the isochronal map (Figure 1B(2) and Movie II). Note that the PP interval between beats (1) and (2) was 355 ms, while the baseline intervals were 340 ms. Because there were irregular cycle lengths and competing pacemaking sites, Figure 1A demonstrates atrial arrhythmias induced by failure of DAD to reach threshold in the SAN. After further increase of isoproterenol dosage to 0.1 μmol/L or

more, the superior SAN became a consistent leading pacemaker site. The DAD-like activity and atrial arrhythmia were no longer observed.

Alternans Between Subthreshold DAD and Sinus Beat

One RA had 17 episodes of alternating subthreshold DAD. All episodes showed alternans of cycle length and the origin of activation. Figure 3 shows a typical example. The pseudo ECG shows PP intervals alternating between 415 ms and 455 ms. During the shorter PP intervals, LDCAE was observed at superior SAN (arrows in Figure 3A) and successfully induced action potentials from that site. The isochronal maps in Figure 3B(1) confirmed that the origin of activation was the superior SAN. However, during longer PP intervals, the subthreshold DAD of SAN (asterisks in Figure 3A) failed to trigger a sinus beat. The isochronal map in Figure 3B(2) shows that the activation started from the inferior SAN.

Subthreshold DADs Causing Atrial Arrhythmia

Figure 4 shows additional examples of subthreshold DADs causing atrial arrhythmia. Figure 4A shows intermittent pattern of subthreshold DAD (asterisks) recorded from the first RA, while figure 4B shows alternans between subthreshold DADs and sinus beats recorded from a second RA. In all 37 different episodes, cycle length prolongation (mean ± SD, 37 ± 25 ms) occurred because these subthreshold DADs failed to trigger sinus beats. The coupling interval of subthreshold DAD was longer than the preceding normal beats (407 ± 48 ms vs. 371 ± 44 ms, p=0.002). A latent pacemaker from outside of the mapped region or inferior RA then antidromically activated the SAN. In some episodes (arrows in Figure 4B), the Ca_i elevation was associated with small or no V_m changes. Figure 4C shows that subthreshold DAD (#) and arrhythmia were recorded only with an intermediate dose of isoproterenol (0.03 μmol/L). Higher and lower doses of isoproterenol resulted in normal sinus rhythm, with the superior SAN as the leading pacemaker site. This is the case for all RAs studied.

Amplitudes and Slopes of Subthreshold DADs

In all 37 episodes of subthreshold DADs, the slopes of DD preceding sinus beats were significantly higher than those of subthreshold DADs (1.7 ± 0.7 AU/s, vs. 1.3 ± 0.6 AU/s, p=0.02, Figure 5A). The slopes of LDCAE preceding sinus beats were also significantly higher than that of subthreshold DADs (4.8 ± 1.4 vs. 3.7 ± 1.3 AU/s, p=0.001, Figure 5B). The maximum amplitude of DD preceding sinus beats was significantly higher than that of subthreshold DADs (0.23 ± 0.04 AU vs. 0.20 ± 0.05 AU, p=0.002, Figure 5C). The maximum amplitude of LDCAE preceding sinus beats was significantly higher than that of subthreshold DADs (0.48 ± 0.07 AU vs. 0.41 ± 0.08 AU, p=0.001, Figure 5D). These findings suggest that insufficient magnitudes of LDCAE and DD were associated with the failure of subthreshold DAD to trigger a full action potential.

Discussion

Major findings

We found that sympathetic stimulation increased heart rate, and induce LDCAE at the superior SAN. In normal conditions, the LACAE induced DD with sufficient V_m changes, leading to the activation of $I_{Ca,L}$ and a full action potential. However, if the LACAE and DD were too small to trigger an action potential, then a DAD is observed.

Subthreshold DADs in the SAN

We propose that the subthreshold DD in the SAN documented in the present study were subthreshold DADs. The reasons were: (A) In all instances, the onset of Ca_i elevation preceded or was simultaneous with the onset of V_m elevation, suggesting non-voltage gated

SR Ca release is the driving mechanism for V_m changes. (B) In some episodes, apparent Ca_i elevation at the SAN was not associated with significant V_m changes. These latter findings are consistent with recent studies that show Ca waves in multicellular preparations may fail to change V_m due to electrotonic interaction with neighboring cells.[14, 17] Single cell transmembrane potential recordings in the past have shown spontaneous membrane potential elevations during late diastole in cells with spontaneous phase 4 depolarizations.[18–19] However, those authors did not perform simultaneous Ca_i transient mapping at the site of V_m changes. It is therefore unclear if those depolarizations were subthreshold DADs or were due to other mechanisms.

SAN Activations and Propagation

Fedorov et al[20] showed that SAN activation exit to the RA through specific pathways. Due to structural complexities, the authors propose that optical signals from the SAN may be mixed with that from the RA. When exit block occurs, the SAN may activate alone. The size of the SAN activation recorded in their study is similar to the large subthreshold DADs observed in the present study. Therefore, an alternative explanation to our observation is that the subthreshold DADs observed in the present study were in fact a full activation of the SAN. Some of the SAN activations failed to propagate into the neighboring RA, resulting in an isolated activation confined to SAN itself.

However, this alternative explanation did not satisfactorily explain all findings of this study. While Fedorov et al[20] did not have simultaneous Ca_i mapping in their study, we observed that spontaneous Ca_i elevation preceded the changes of the DAD-like V_m changes at the SAN. Due to this temporal sequence, the Ca_i elevation could not have been triggered by the Vm changes. A second inconsistency is that the LDCAE amplitude and slope were larger in conducted beats than in the ones not associated with conducted beats, as shown in Figure 4. In some cases, the Ca_i elevation was not associated with any changes of the V_m (arrows, Figure 4B). Exit block hypothesis cannot fully explain the variations of V_m amplitude in the SAN, nor the LDCAE without V_m changes at that site. On the other hand, LDCAE with variable V_m changes are commonly observed in the intact ventricular tissues and in the whole heart.[14, 17] Therefore, while the results of the present study can be partially explained by the exit block hypothesis,[20] we propose that subthreshold DAD of SNA remains the best explanation of our findings.

Subthreshold DAD and a New Mechanism of Atrial Arrhythmia

An escape beat after a subthreshold DAD in a single SAN cell was followed by a full compensatory pause.[12] In intact RA preparation, however, failure of subthreshold DAD to reach threshold allowed latent pacemakers elsewhere to activate the atrium, resulting in atrial arrhythmia. In these arrhythmic episodes, a beat that closed the longer PP interval, rather than a premature beat, was from an ectopic focus. This phenomenon was also compatible with the concept of parasystole in which the SAN was a source of normal rhythm while the ectopic pacemaker was the parasystolic focus. When the SAN failed to generated a rhythm to inhibit (or pre-excite) the parasystolic focus, the latter was able to exit and capture the entire RA. Shinohara et al[21] recently used the same intact RA preparation to study the mechanisms of pacemaking of the ectopic pacemakers. They found that while spontaneous SR Ca release (Ca clock) underlies isoproterenol-induced increase of superior SAN activity, the atrial ectopic pacemaker is less dependent on the Ca clock and more dependent on the membrane clock for its automaticity. These ectopic pacemakers outside the SAN therefore can effectively serve as backup pacemakers when SAN fails. The co-existence of two pacemaking sites resulted in atrial arrhythmias observed in the present study.

Clinical Implications

Atrial arrhythmia occurs in 35% of apparently normal subjects during treadmill exercise testing.[22] It was possible that some of these atrial arrhythmias were induced by the mechanisms reported in the present study. While atrial arrhythmias might be benign in normal subjects, exercise induced atrial arrhythmias were known to be associated with adverse long-term outcome in patients with underlying cardiovascular diseases.[23] It was possible that these arrhythmias were manifestations of abnormal SR function associated with organic heart diseases. Abnormal SR function could affect both cardiac contraction and the Ca clock of the SAN. The association between exercise induced atrial arrhythmia and poor prognosis might therefore be an epiphenomenon related to poor SR Ca handling in those patients.

Study limitations

Because it was not possible to record the optical signals continuously for prolonged period of time, some subthreshold DADs might not have been recorded. Therefore, the low incidence of subthreshold DAD in the intact SANs (4/31, or 13%) shown in this study may underestimate the importance of this mechanism in atrial arrhythmogenesis.

Conclusion

The isoproterenol-induced LDCAE of superior SAN results in DD and full action potentials in most cases. However, in rare situations, the isoproterenol-induced LDCAE triggered only a DAD but not an action potential, allowing latent ectopic atrial pacemakers to activate and excite the atrium. The failure of DAD-like DD to consistently trigger a sinus beat is a novel mechanism of atrial arrhythmogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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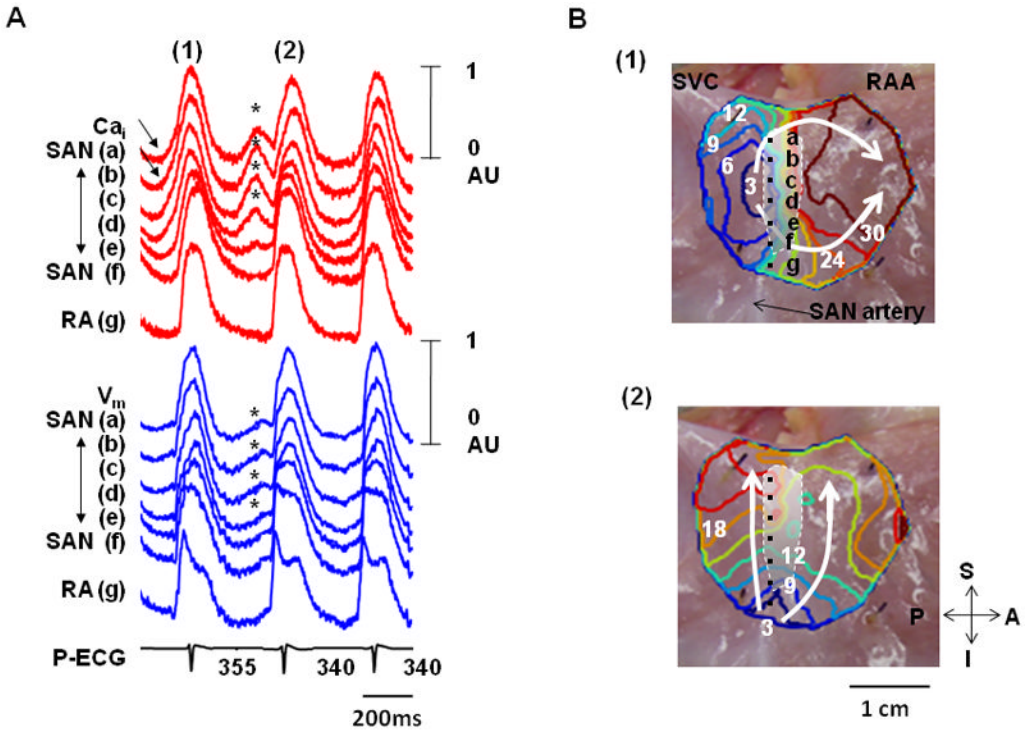

Figure 1. The intermittent pattern of subthreshold DADs

These tracings were obtained during isoproterenol infusion (0.03 μmol/L) in the first RA preparation. A, Optical signals of Ca_i (red) and V_m (blue) from superior (a, b), middle (c, d), and inferior (e, f) SAN, and RA (g). There were 3 consecutive activations in this figure. Among them, the first (1) and third beats show LDCAE (arrows) followed by the initiation of sinus beats from the same sites. In contrast, the second beat (2) showed both LDCAE on Ca_i tracings and subthreshold DADs on V_m tracings (asterisks). The down slope of the subthreshold DADs were observed because they failed to trigger an action potential. B, V_m isochronal maps of the first (1) and second (2) beats. The white shaded area is the SAN. The first beat (1) was from SAN. Because subsequent LDCAE in the SAN (asterisks in Panel A) failed to trigger a sinus beat, an ectopic pacemaker was able to take over and activate the mapped region (2). RAA, right atrial appendage; SVC, superior vena cava; A, anterior; P, posterior; S, superior; I, inferior.

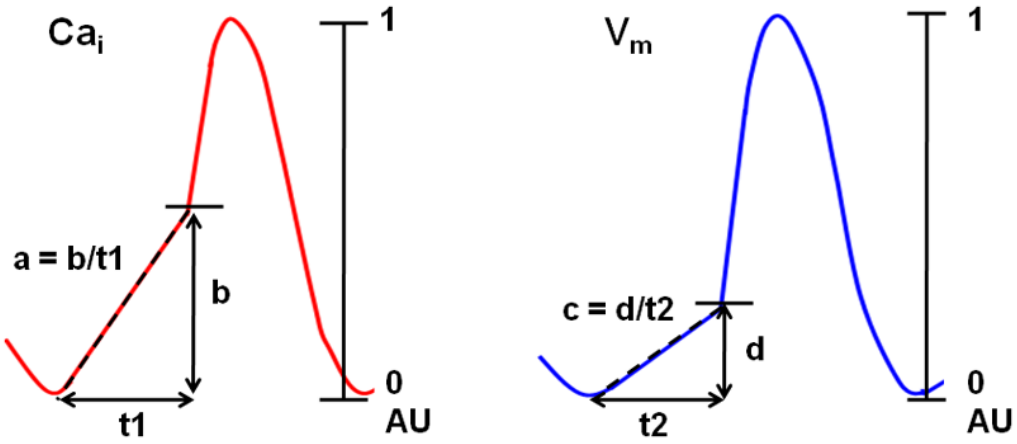

Figure 2. The methods of measurement

Schematic explanation of how the slope of LDCAE (a) and DD (c), and the maximum amplitudes of LDCAE (b) and DD (d) were measured. The largest magnitude of Ca_i and V_m changes was used as 1 arbitrary unit (AU). The slopes of segments a and c were calculated by b/t1 and d/t2, respectively. The unit of the slope was AU/s. In this and subsequent figures, red lines indicate Ca_i tracings, while blue lines were used for V_m tracings.

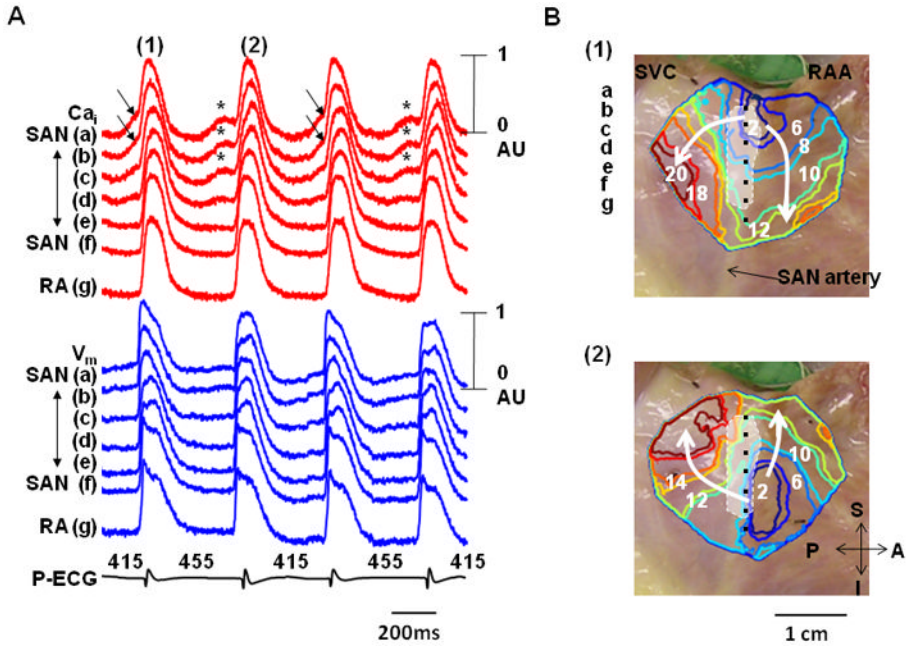

Figure 3. The alternans pattern of Ca_i release and the development of cardiac arrhythmia

These data were obtained during isoproterenol infusion (0.3 μmol/L) in the second RA. A, Ca_i (red) and V_m (blue) tracings from superior (a, b), middle (c, d), and inferior (e, f) SAN, and RA (g). Arrows point to LDCAE that induced sinus beats from SAN. Asterisks indicate LDCAE that did not result in a sinus beat. B, V_m isochronal maps. The first beat (1) originated from SAN while the second beat (2) originated from an ectopic pacemaker. The white shaded area is the SAN. These two activation patterns alternated with each others, resulting in a cycle length alternans in Panel A.

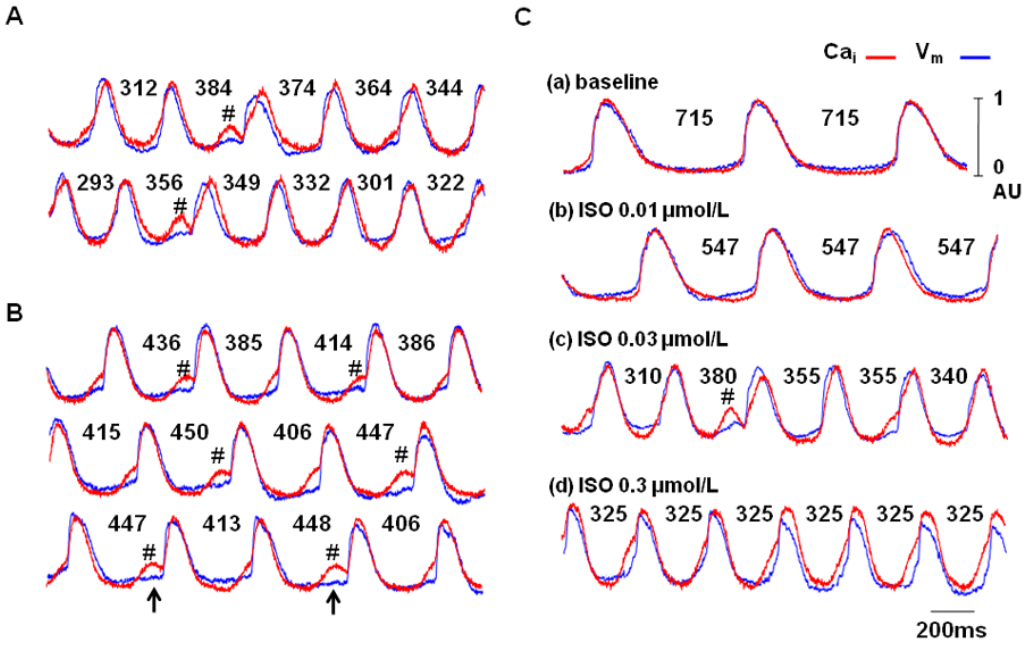

Figure 4. Additional examples of the subthreshold DADs

Ca_i (red) and V_m (blue) tracings of superior SAN during isoproterenol infusion from the first (A) and second RA preparation (B). Note that there were intermittent lengthening of the activation cycle lengths. The presence of LDCAEs and subthreshold DADs (asterisks) were coincidental with the prolongation of the cycle lengths. Upward arrows indicate that occasionally, large LDCAEs were not associated with changes of V_m. C shows the effects of isoproterenol dosage on the occurrence of the subthreshold DADs (asterisks). The subthreshold DADs only occurred with intermediate dose (0.03 µmol/L) of isoproterenol. Higher and lower doses did not induce LDCAE or subthreshold DADs. The numbers are cycle lengths in ms.

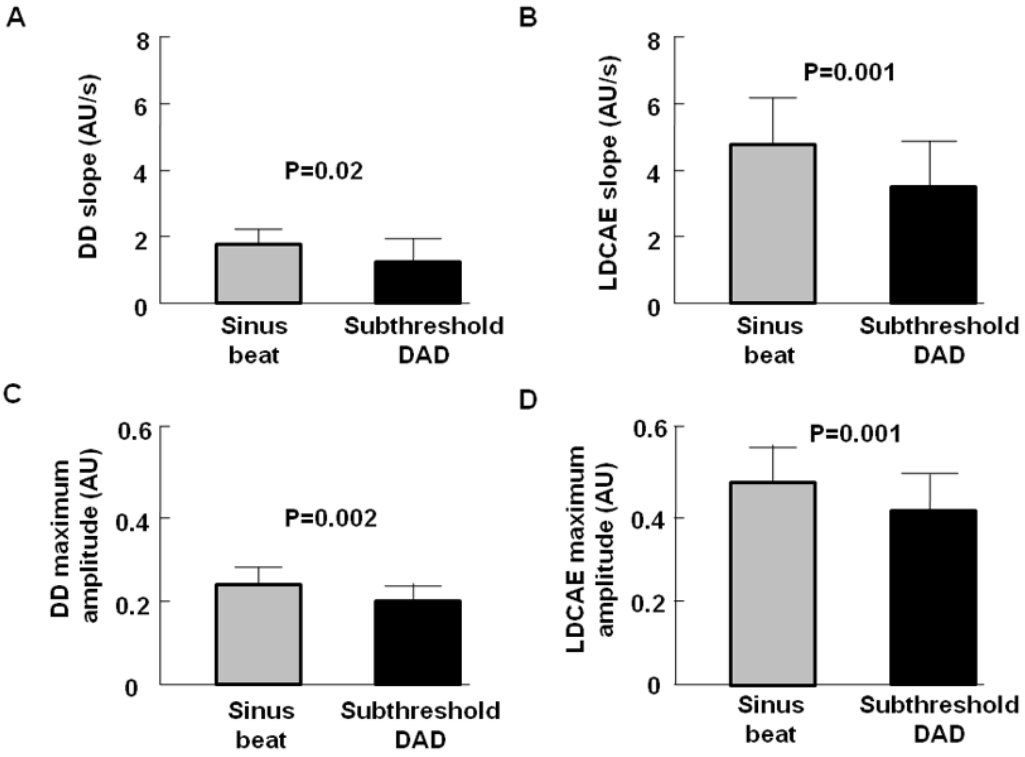

Figure 5. The comparisons between subthreshold DADs (n=37) and sinus beats (n=37)

A, The slopes of DD. B, The slopes of LDCAE. C, The maximum amplitude of DD. D, The maximum amplitude of LDCAE. These data show that the slopes of DD and LDCAE were higher for the sinus beats than the subthreshold DADs. Similarly, the maximum amplitudes of DD and LDCAE were higher in sinus beats than for subthreshold DADs. These findings suggest that insufficient magnitudes of LDCAE and DD were associated with the failure of subthreshold DAD to reach threshold.