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Calcium transient dynamics and the mechanisms of ventricular vulnerability to single premature electrical stimulation in Langendorff-perfused rabbit ventricles

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Abstract

BACKGROUND—Single strong premature electrical stimulation (S_2) may induce figure-eight reentry. We hypothesize that Ca current-mediated slow-response action potentials (APs) play a key role in the propagation in the central common pathway (CCP) of the reentry.

METHODS—We simultaneously mapped optical membrane potential (V_m) and intracellular Ca (Ca_i) transients in isolated Langendorff-perfused rabbit ventricles. Baseline pacing (S_1) and a cathodal S_2 (40 – 80 mA) were given at different epicardial sites with a coupling interval of 135 ± 20 ms.

RESULTS—In all 6 hearts, S_2 induced graded responses around the S_2 site. These graded responses propagated locally toward the S_1 site and initiated fast APs from recovered tissues. The wavefront then circled around the refractory tissue near the site of S_2 . At the side of S_2 opposite to the S_1 , the graded responses prolonged AP duration while the Ca_i continued to decline, resulting in a Ca_i sinkhole (an area of low Ca_i). The Ca_i in the sinkhole then spontaneously increased, followed by a slow V_m depolarization with a take-off potential of -40 ± 3.9 mV, which was confirmed with microelectrode recordings in 3 hearts. These slow-response APs then propagated through CCP to complete a figure-eight reentry.

CONCLUSION—We conclude that a strong premature stimulus can induce a Ca_i sinkhole at the entrance of the CCP. Spontaneous Ca_i elevation in the Ca_i sinkhole precedes the V_m depolarization, leading to Ca current-mediated slow propagation in the CCP. The slow propagation allows more time for tissues at the other side of CCP to recover and be excited to complete figure-eight reentry.

Keywords

Action potentials; Reentry; Mapping; Ventricular fibrillation; Electrical stimulation

Introduction

A single strong premature electrical stimulus (S_2) can induce ventricular fibrillation (VF) in normal hearts. Computerized mapping studies showed that when the S_1 and the S_2 were given at different sites with the line connecting S_1 and the S_2 parallel to the fiber orientation, the S_2 may induce a figure-eight pattern that degenerates into VF.¹ A critically timed second premature stimulus (S_3) given at the central common pathway (CCP) can abort the formation of figure-eight reentry and VF.² Unlike other parts of the reentrant circuit, the CCP of this figure-eight pattern demonstrates a very slow propagation velocity (12.5 cm/s) as compared with 52 cm/s to 70 cm/s after S_1 stimulation.³ The slow propagation creates a time delay, which allows the cells at the other end of the CCP to fully recover and be excited again (reentry). The mechanisms of slow propagation within the CCP are unclear. With the advancement of optical mapping techniques, it is now possible to study the membrane potential (V_m) and intracellular Ca (Ca_i) simultaneously with a high spatial resolution. We hypothesize that Ca current-mediated slow-response action potentials (APs) are in part responsible for the mechanisms of figure-eight reentry induced by a single strong S_2 . The purpose of the present study was to perform simultaneous dual V_m and Ca_i mapping in Langendorff-perfused rabbit ventricles to test this hypothesis.

Methods

The research protocol was approved by the Institutional Animal Care and Use Committee.

Tissue preparation

New Zealand White rabbits weighing 3–5 kg were anesthetized, and the hearts were quickly removed. The ascending aorta was cannulated and secured for retrograde perfusion with warm ($36.5^\circ\text{C} \pm 0.5^\circ\text{C}$) and oxygenated Tyrode solution at a rate of 30 to 40 mL/min. The composition of the Tyrode solution (in mmol/l) at a pH of 7.4 ± 0.05 was NaCl 125, KCl 4.5, NaH_2PO_4 1.8, NaHCO_3 24, CaCl_2 1.4, MgCl_2 0.5, glucose 5.5, and albumin 50 mg/l in deionized water. The coronary perfusion pressure was regulated and maintained at 60 cm H_2O .

Optical mapping

Six hearts were used for simultaneous dual optical mapping of V_m and Ca_i .⁴ The emitted fluorescence was filtered through a 585 ± 20 nm band-pass filter for the Ca_i signal recording and a 710-nm long-pass filter for V_m recording to prevent cross-talk. The fluorescence was then acquired simultaneously with 2 charge-coupled device cameras (CA-D1-0128T, Dalsa Inc. Waterloo, Canada) at 4 ms/frame. The digital images (128×128 pixels) were gathered from the epicardium of the left ventricle (20×20 mm² area), resulting in a spatial resolution of 0.16×0.16 mm² per pixel. We acquired 1,000 frames continuously with 12-bit resolution. Optical signals were processed with both spatial (3×3 pixels) and temporal (3 frames) filtering. We used a grid to calibrate the field of view of these 2 cameras. The locations were then mathematically matched so that we could analyze the V_m and Ca_i at the same recording sites. Cytochalasin D (5 $\mu\text{mol/l}$, Sigma, St. Louis, MO) was used to inhibit contraction.

Construction and interpretation of 2-dimensional maps

The ratio maps were used to examine V_m activation and changes in Ca_i levels. The average fluorescence level ($\bar{F}$) of the entire data window was first calculated. The ratio is then calculated as $(F - \bar{F})/\bar{F}$. We assigned shades of red to represent above-average fluorescence and shades of blue to represent below-average fluorescence to generate the ratio maps. The phase maps were constructed using a time-delay embedding method.⁵ Phase singularity points are sites where neighboring elements show a continuous progression of phases from $-\pi$ to $+\pi$.

We performed 3 additional studies to record single-cell transmembrane potential using standard glass microelectrodes. The recording sites were at the side of S_2 opposite to the S_1 site. Transmembrane potential recordings were digitized at 5 KHz.

Stimulation protocol

Two stainless steel stimulating electrodes (tip diameter 0.20 mm) were placed on the left ventricle: one for baseline pacing (S_1) at a cycle length of 350 ms and another for S_2 . The line connecting S_1 and S_2 was parallel to the fiber orientation. After 8 S_1 , a unipolar cathodal S_2 (10-ms pulse width) was applied with currents ranging from 10 to 100 mA in increments of 10 mA. The S_2 stimuli were given during the relative refractory period to induce reentry.

Effects of verapamil

An additional 5 rabbits were studied to determine the effects of verapamil on the patterns of reentry. After baseline studies, verapamil at concentrations of 0.5, 1, 2, 4, 8, and 16 $\mu\text{mol/l}$ ($N = 3$) or at a concentration of 100 $\mu\text{mol/l}$ ($N = 2$) were given and the stimulation protocol was repeated.

Data analysis

The results are presented as mean $\pm$ standard deviation. Analyses of variance with post hoc analysis (Scheffe tests) were used for multiple comparisons and t test was used to analyze the differences of the means. The null hypothesis was rejected for $P \leq .05$.

Results

Figure-eight excitation pattern and VF were induced and mapped in the first 6 rabbits studied. The vulnerable windows were between 90 and 170 ms (mean 141 ± 14 ms). The interval between S_2 and the first activation at the exit of the CCP was 150 ± 14 ms. The thresholds for the induction of reentry and VF were 33 ± 6 mA and 61 ± 13 mA, respectively. A total of 413 episodes of stimulation (59 ± 35 episodes per rabbit) were analyzed. The S_2 resulted in virtual electrode polarization and graded responses near the S_2 site, followed by figure-eight reentry. We measured the conduction velocity of S_1 activation and the APD_{90} (AP duration at 90% of repolarization) at the S_1 and S_2 sites. Conduction velocity (77.8 ± 13.9 cm/s) and APD (S_1 site 165.3 ± 11.5 ms, S_2 site 162.7 ± 12.8 ms) at the beginning of the experiment did not differ significantly from conduction velocity (77.0 ± 13.6 cm/s) and APD (S_1 site 165.3 ± 11.5 ms, S_2 site 162.7 ± 12.8 ms) at the end of the experiment. Figure 1A shows a typical example of V_m and Ca_i patterns after S_1 pacing. The downward direction of the arrows indicates the sequence of consecutive activations at 20-ms intervals. Note that the activation and repolarization near the S_2 site were homogeneous within the first 100 ms after S_2 (left column). Figure 1B shows that the onset of V_m preceded the onset of Ca_i in S_1 paced beat. Figures 1C and 1D show examples of optical recordings near S_2 site over the 27-minute duration of the experiment. These optical signals were stationary throughout the experiment in all rabbits studied.

Dissociated V_m and Ca_i fluorescence after S_2

Although the Ca_i transients in S_1 paced beats followed the rise of V_m , the V_m and Ca_i fluorescence after S_2 was more complicated. We found in all episodes of figure-eight reentry dissociation between V_m and Ca_i transients in a small area close to S_2 site. The dissociation occurred because of continuous and monotonic Ca_i decline during S_2 -induced graded responses. Figure 2 shows examples of V_m and Ca_i signals 76 ms after S_2 . In most of the mapped regions, S_2 induced both graded responses (downward black arrows) associated with transient elevation of Ca_i (upward black arrows). However, at the side of S_2 that was opposite

to the S_1 site (at 4 o'clock location), the graded response was small and did not interrupt the smooth Ca_i decline. Afterward the Ca_i elevation occurred spontaneously before the rise of V_m (asterisk). The top phase plot (blue arrow) showed counterclockwise rotation (white arrows), consistent with V_m depolarization preceding the rise of Ca_i . This is true for most of the mapped region. In contrast, the phase plot at right lower corner showed counterclockwise rotation (white arrows) interrupted by clockwise rotation (yellow arrows), indicating a rise of Ca_i preceding V_m depolarization (Ca_i prefluorescence).

Figure-eight reentry induced by S_2

Figure 3 shows an example of V_m and Ca_i responses after S_2 . An isochronal map of the first 100 ms after S_2 (left panel, Figure 3A) shows the propagation of the graded responses away from S_2 (blue to green colors). Block (between green and red) occurred in the right lower direction due to tissue refractoriness. However, graded response encountered recovered tissue in left upper portion of the mapped region (S_1 direction), eliciting propagated fast AP that circled around the site of S_2 toward the lower portion of the mapped region. The right panel of Figure 3A shows the completion of figure-eight reentry by slow propagation along the CCP (blue color), followed by continuous reentrant excitation (green-yellow-red color).

Figure 3B shows the maps of the same episode. The S_1 and S_2 sites were marked with yellow dots. S_2 was used as time zero. A dog-bone pattern⁶ (between the 2 yellow arrows at 0 ms frame) formed immediately after S_2 . This pattern was followed by graded responses that propagated away from S_2 along the fiber direction. At frame 48 ms, the graded responses between the 2 red arrows encountered refractory tissue. However, the graded responses that propagated toward the S_1 site encountered recovered tissue and elicited fast propagated APs (white arrows, frames 0 and 48 ms). At frame 76 ms, the wavefronts (white arrows) propagate toward the right lower corner while bypassing the areas with graded responses shown in frame 48 ms (red arrows). Because the graded responses was associated with either no or very small increase of Ca_i , the Ca_i level continued to decline, resulting in a large area of blue region in frames 76 ms on the Ca_i map. The size of the blue region then gradually decreased at 104 ms and 120 ms. The final blue region (at 120 ms) was surrounded by a red region, forming a Ca_i sinkhole⁷ (asterisk, frame 120 ms). The corresponding site in the V_m map also showed a V_m sinkhole (frame 104 ms and 120 ms), resulting in a heterogeneous distribution of V_m . The presence of these V_m and Ca_i sink-holes and heterogeneities was characteristic for the induction of reentry and VF in this model. A phase map to the right of panel B shows 2 phase singularities (arrowheads) at frame 72 ms.

Slow-response action potentials from areas with reduced Ca_i fluorescence

The optical signals from 10 evenly spaced sites (1 to 5 and 6 to 10) marked as green dots on frame 104 ms are shown in Figures 3C to 3F. Among them, Figures 3D and 3F show the same pixels as 3C and 3E, respectively, but were arranged to demonstrate the temporal relationship between V_m and Ca_i at the same sites. In panel F, the rise of Ca_i preceded the rise of V_m in site 6. The same was true for panel D, site 3. However, in sites 1 and 2 of panel D, the onset of V_m and Ca_i occurred roughly at the same time. At those sites, V_m during the AP upstroke has a slow rate of rise, compatible with Ca current-mediated activation. The slow-response AP then propagated through the CCP (arrow on V_m map, frame 120 ms) and caused reentry at the left upper corner of the mapped region. Figure 3G shows the actual recording during induction of VF. The V_m and Ca_i amplitudes of S_1 paced beats were used as calibrations for determining the V_m and Ca_i fluorescent levels after the induction of VF.

Characteristics of a central common pathway

Figures 4 and 5 show another example of figure-eight reentry induced by S_2 . In this example, we show both the area with slow-response V_m initiation and the slow propagation in the CCP

in greater detail (Figure 4). (The optical signals along the green arrows in Figure 4B, frames 68 ms and 136 ms, were displayed in Figure 5.) Figure 4A shows 2 consecutive isochronal maps of figure-eight reentry. Figure 4B shows the direction of wavefront propagation (white arrows) after S_2 , illustrating a dog-bone pattern (frame 0 ms, between yellow arrows), the propagated graded responses (frame 28 ms), and the formation of V_m and Ca_i sinkholes (frames 68 ms and 88 ms). These sinkholes (blue) were then filled by red color (frame 116 ms) followed by slow propagation in the CCP (frame 136 ms) and reentry (frame 152 ms).

Figure 5A shows that an S_2 stimulus induced graded responses (downward black arrows) in V_m channels 1 to 5, which were the sites surrounding S_2 . These graded responses elevated the V_m in cells between sites 1 to 5. In the meantime, the Ca_i (red) continued to decline (site 1) or showed only small elevation (site 2), resulting in dissociation between the V_m recovery and the Ca_i reuptake or extrusion in those sites. Figure 5B shows overlapping fluorescence of V_m and Ca_i from the same tracings shown in Figure 5A. Arrows point to spontaneous rise of Ca_i fluorescence that either preceded the onset of V_m (sites 3 and 4, arrows) or occurred simultaneously with the onset of V_m (site 1). The V_m at those sites showed slow responses. Assuming that the membrane potential ranged from -75 mV to $+15$ mV during S_1 pacing (Figure 3G), the take-off V_m of the slow-response AP at the side of S_2 opposite to the S_1 site averaged -40 ± 3.9 mV.

Figures 5C and 5D show examples of V_m and Ca_i signals within the CCP (sites 6–10, as marked in Figure 4, frame 136 ms). For all episodes analyzed, the estimated take-off V_m in the CCP averaged -27 ± 2.5 mV during reentry. This V_m is much higher than the diastolic V_m at the same site (-71.2 ± 1.6 mV) during S_1 pacing ($P < .0001$). The propagation velocity from the entrance to the exit of the CCP was 19.2 ± 1.9 cm/s. This velocity is much slower than the propagation velocity during S_1 pacing at the same site (73.4 ± 8.6 cm/s, $P = .0002$). The V_m signals within the CCP (Figures 5C and 5D) show slow rising phase 0. For all episodes analyzed, the average phase 1 dV/dt of CCP in a reentrant circuit (1.8 ± 0.5 V/s) was significantly ($P < .01$) lower than the dV/dt at the same site during S_1 pacing (6.0 ± 1.1 V/s). Furthermore, the onset of V_m and the Ca_i were nearly simultaneous in sites 6 to 10. These findings suggest Ca current-mediated propagation, as Na channels are inactivated at these voltage ranges. Figure 5E shows optical signals from the exit sites of the CCP (sites 11 and 12 of Figure 4B). The V_m shows rapid upstroke, consistent with Na-mediated activation.

Transmembrane potential evidence of slow-response V_m near the entrance of CCP

We performed transmembrane potential recordings in 3 rabbits by impaling a cell at the side of S_2 opposite to the S_1 site. Figure 6A shows a single cell transmembrane potential recording after an 80-mA S_2 given 130 ms after the last S_1 . The S_2 -induced graded response (arrow “g”) slightly lengthened the APD but did not induce reentry. In contrast, an 80-mA S_2 given 170 ms after the last S_1 induced graded response (“g” in Figure 6B) followed by a slowly rising depolarization (asterisk). The take-off potential of this slow AP was -46 mV with a maximum dV/dt of 17 V/s. Figure 6C shows another recording with a 100-mA S_2 given 170 ms after the last S_1 induced 3 repetitive responses. The depolarization after the S_2 in this episode is also slow, with $(dV/dt)_{\max}$ of 22 V/s (asterisk). These single-cell transmembrane potential recordings were similar to the optical V_m signals shown at site 1 of Figure 3A, and indicate that the high take-off potentials recorded with optical dyes were not due to light scattering from underlying tissue. We observed a total of 36 episodes of slow-response AP in 3 rabbits studied, including 10 episodes that were followed by 1 to 2 repetitive responses. The $(dV/dt)_{\max}$ of the slow-response V_m in all episodes averaged 19 ± 6 V/s.

Sinkholes and vulnerability

We analyzed V_m and Ca_i snapshots 80 ms after the S_2 at various coupling intervals and stimulus strength. Figure 7 shows such snapshots in one rabbit. We found that repetitive responses were induced only when apparent V_m and Ca_i sinkholes were observed. This phenomenon was present in all 3 rabbits in which both upper and lower limits of vulnerability were present. When the shock strength was either above the upper limit or below the lower limit of vulnerability, an apparent Ca_i sinkhole was not observed. We then analyzed the Ca_i and V_m fluorescent levels in the Ca_i sinkhole in episodes with repetitive responses or VF and in the same sites in episodes without either repetitive response or VF. The Ca_i fluorescence in the Ca_i sinkhole in former episodes (0.41 ± 0.03) was significantly ($P < .01$) less than the Ca_i fluorescence in latter episodes (0.57 ± 0.07). The V_m within the V_m sinkhole was also lower in former episodes (-21.4 ± 3.8 mV) than in latter episodes (-10.2 ± 5.2 mV, $P < .01$). We also calculated the ratio of Ca_i fluorescence between the center of the sinkhole and the edge of the sinkhole, and call that the spatial gradient of Ca_i . We found that the spatial gradient of Ca_i was significantly higher in former episodes (0.35 ± 0.06) than in the latter episodes (0.08 ± 0.05 , $P < .01$).

Effects of verapamil

Figure-eight reentry could still be induced at verapamil concentrations of 0.5, 1, 2, and 4 $\mu\text{mol/l}$. When the concentration was increased to ≥ 8 $\mu\text{mol/l}$, no figure-eight reentry was induced. Rather, the S_2 induced focal discharges from areas around the S_2 .

Discussion

We mapped the V_m and Ca_i transients after a single strong S_2 given to an epicardial electrode. The results showed that on the side of S_2 that is opposite to S_1 , a critically timed S_2 stimulus was associated with graded responses and APD prolongation without interrupting the monotonic decline of the Ca_i transient. The V_m and Ca_i dissociation was followed by the formation of V_m and Ca_i sinkholes at that site. These areas were the sites for later development of slow-response APs that propagated slowly through the CCP. The slow propagation allowed time for the cell on the other site of the CCP to recover, leading to reentrant excitation.

The nature of slow-response action potentials after S_2

Several lines of evidence suggest that the slow propagation in the CCP is mediated by I_{Ca-L} rather than I_{Na} . (1) The take-off V_m in the CCP was high. At that level of V_m , I_{Na} is inactivated, leaving I_{Ca-L} the most likely membrane current responsible for propagation. (2) The propagation velocity from the entrance to the exit of the CCP was much slower than the propagation velocity during S_1 pacing. (3) The average dV/dt in the CCP was much lower than during S_1 pacing. (4) Ca_i prefluorescence or a simultaneous rise of V_m and Ca_i was present in the CCP. The presence of Ca_i prefluorescence at the entrance of the CCP suggests early afterdepolarization (EAD) as a possible mechanism of impulse initiation at that site.⁸ One possible mechanism of EAD after S_2 is the recovery of I_{Ca-L} from inactivation. Computer simulation studies⁹ showed that APD prolongation gives time for I_{Ca-L} to recover from the previous activation and reactivates, leading to EAD. The same mechanism might be applicable to the sinkhole where APD was prolonged by S_2 but the Ca_i continued to decline, suggesting progressively increased the I_{Ca-L} availability. When the I_{Ca-L} sufficiently recovers, it becomes available for reactivation.

A second possible mechanism for the slow response from the Ca_i sinkhole is the spontaneous sarcoplasmic reticulum Ca release. The dark blue color in the Ca_i sinkhole implies a low Ca_i concentration due to more complete sarcoplasmic reticulum uptake and Ca_i extrusion by the

Na-Ca exchanger. If sarcoplasmic reticulum Ca is full, spontaneous Ca release might occur, leading to EADs and automaticity.¹⁰

A third possible explanation for the slow-response APs is the electrotonic interaction with surrounding cells. Due to the bidirectional coupling between Ca_i and APD,¹¹ heterogeneous Ca_i levels after S_2 may enhance local gradients in refractoriness. Rabbit ventricles typically show positive Ca_i -APD coupling, so that a larger Ca_i transient prolongs APD by enhancing inward Na-Ca exchange current during the plateau phase and a smaller Ca_i transient shortens the APD.¹¹ At the V_m sinkholes, the APD in the sinkhole was shorter than the surrounding sites. Electrotonic currents from the surrounding depolarized regions might assist impulse formation in the sinkhole. This mechanism is similar to phase-2 reentry or reflection.¹² We propose that this mechanism most likely explained the association between the sinkhole and the repetitive responses after S_2 .

Strong electrical stimulation and Ca_i transients

Electrical shocks may cause both positive and negative inotropic effects.¹³ More recent study¹⁴ demonstrated that the timing of the shock relative to action potential is an important determinant of its effects on Ca_i transients. It is possible that strong current flow near the S_2 site was responsible for the creation of a Ca_i sinkhole by selectively depressing Ca_i transient in the center of the sinkhole. However, the formation of the sinkhole was not at the site of S_2 . Rather, it was a few millimeters away from the S_2 site when the field strength was expected to be lower than that at the site of S_2 . The occurrence of a Ca_i sinkhole in those areas suggests that the sinkhole cannot be explained solely by the shock-induced suppression of Ca transients.

Study limitations

The spatial registration algorithm used in this study aligned the epicardial locations of V_m and Ca_i recordings. Such an alignment may not be perfect due to different tissue optical properties at different wavelengths of the V_m and Ca dyes.¹⁵

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Figure 1. Stability of V_m and Ca_i during the study. **A:** Sequential maps of S_1 activation. **B:** V_m preceded Ca_i during S_1 pacing. **C and D:** Temporal stability of V_m and Ca_i , respectively, from the beginning (0 min) to the end (27 min) of experiment at a site near S_2 . Traces marked by asterisks were obtained from the same episode as in A.

Figure 2.

Dissociation of V_m and Ca_i responses to S_2 . This figure shows the V_m and Ca_i relationship following S_2 at an S_1 – S_2 coupling interval of 140 ms. The V_m map shows a snapshot taken 76 ms after S_2 . This V_m map was used to indicate the location of the optical signals relative to the S_1 and S_2 sites. We included 2 phase plots (blue arrows) of Ca_i versus V_m for the entire duration of the tracing. The phase plot on the right lower edge showed initial counterclockwise rotation (white arrows) interrupted by a period of clockwise rotation (yellow arrows). The clockwise rotation corresponded to the rise of Ca_i before the rise of V_m at the site of S_2 opposite to the site of S_1 .

Figure 3. Dual mapping of figure-eight reentry. **A:** Consecutive isochronal maps of figure-eight reentry induced by a 40-mA cathodal S_2 given 150 ms after the last S_1 . **B:** Optical ratio map of the wavefront propagation after S_2 . **C–F:** Actual optical signals at sites marked on the frame 104 ms.

Figure 4.

V_m and Ca_i maps of figure-eight reentry. **A:** Isochronal maps after S₂, with the time of S₂ as time zero. **B:** The fluorescent ratio ($\Delta F/F$) maps of the same episode. White arrows indicate direction of wavefront propagation. Yellow arrows indicate area with graded responses. Green arrow indicates the sites whose optical signals are displayed in Figure 5.

Figure 5.

Ca_i prefluorescence and slow conduction in the CCP. These data are from the same episode as shown in Figure 4B. The V_m and Ca_i in the sinkhole (sites 1–5) and the CCP (sites 6–10) are shown. **A:** Five optical signals of V_m and Ca_i along the path of 1→5. **B:** The same 5 optical signals with V_m and Ca_i overlapping with each other. **C:** The V_m and Ca_i separately, whereas **D** shows the same data with V_m overlapping with Ca_i . **E:** The signals from sites 11 and 12 in Figure 4B.

Figure 6.

Transmembrane potential recordings with glass microelectrode. **A:** S_2 applied at the early phase 3 induced a graded response (g) but no reentry. **B:** S_2 induced graded response (g), followed by a slow rise of V_m (asterisk). This slow rise of V_m is morphologically consistent with EAD. However, it can also be due to I_{Ca-L} -mediated slow-response AP. Both phenomena are expected to have a low take-off potential as shown in this recording. **C:** induced slow-response AP followed by repetitive S_2 activations. APD₇₀ (APD at 70% of repolarization) was prolonged by the S_2 in all 3 examples.

Figure 7.

Ca_i sinkhole and vulnerability. Optical snapshots were obtained 80 ms after S_2 in all episodes. Figure-eight reentry or repetitive ventricular responses were induced when obvious sinkholes were observed. When sinkholes were less apparent, no repetitive response or VF was induced. No sinkhole or reentry was seen when the coupling interval was >160 ms or <140 ms in this rabbit (not shown). Green squares encircle episodes of figure-eight reentry without subsequent degeneration of VF. The numbers on top of the V_m and Ca_i panels show V_m (mV) and Ca_i fluorescent levels inside the sinkholes, respectively. The number on the bottom of the Ca_i panel shows the ratio between the Ca_i fluorescence inside and outside the sinkholes.