Importance of spatiotemporal heterogeneity of cellular restitution in mechanism of arrhythmogenic discordant alternans

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BACKGROUND Spatially discordant cellular alternans form a substrate for development of unidirectional block and ventricular fibrillation. However, the mechanisms responsible for discordant alternans remain poorly understood. Previous work suggests electrical restitution is critical to the development of alternans in single cells.

OBJECTIVES The purpose of this study was to investigate the hypothesis that spatial and temporal heterogeneities of restitution underlie the mechanism eliciting discordant alternans.

METHODS Steady-state pacing was used to elicit concordant cellular alternans in nine Langendorff-perfused guinea pig hearts. A single extrastimulus (S2) was applied every 51st beat following either the even or the odd beat of alternans. The cellular response to S2 was determined using optical mapping to generate action potential duration (APD) restitution curves from 256 ventricular sites for both the even and the odd beats.

RESULTS Restitution kinetics were temporally heterogeneous during alternans, as restitution curves between the even and the odd beats differed significantly. Temporal heterogeneity was quantified by the average separation of restitution between the two curves, or Δ-restitution. Δ-Restitution was spatially heterogeneous and proportional to the amount of alternans at a given ventricular site. A computer simulation based on the experimental results showed the mechanism of discordant alternans was dependent on both spatial and temporal heterogeneities of restitution.

CONCLUSION Both temporal and spatial heterogeneities of restitution exist during cellular alternans in the intact heart. Temporal heterogeneities of restitution, quantified by Δ-restitution, are proportional to the magnitude of cellular alternans. The combination of spatial and temporal heterogeneities of restitution may underlie the genesis of discordant alternans.

KEYWORDS Repolarization; Reentry; Alternans; Optical mapping; Arrhythmias

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Beat-to-beat alternations of the ECG T wave (i.e., T-wave alternans [TWA]) is closely associated with electrical instability in the heart.[1–4] We previously found that TWA was caused by primary alternations in cellular repolarization.[5] Furthermore, TWA was mechanistically linked to initiation of ventricular arrhythmias by spatially discordant cellular alternans. During discordant alternans, ventricular action potentials from neighboring cells alternated with opposite phase, which created steep spatial gradients of repolarization necessary for development of functional block and ventricular fibrillation.[5] Although the arrhythmogenicity of discordant alternans is clear, the mechanisms underlying the generation of discordant alternans are poorly understood.

One factor that has been implicated in the mechanism of cellular alternans is electrical restitution. Restitution is defined as the modulation of action potential duration (APD) in response to a premature stimulus. Restitution has explained the development of transient action potential alternans in Purkinje fibers.[6] Furthermore, nonmonotonic (e.g., biphasic) restitution curves can cause action potential oscillations occurring at the alternans frequency as well as other frequencies.[7] The "restitution hypothesis" states that cellular alternans will occur when the slope of the APD restitution curve exceeds unity.[8] However, emerging experimental evidence suggests that action potential alternans is more likely explained by sarcoplasmic reticulum calcium handling than by restitution properties.[9–11] In contrast to its uncertain role in generating alternans on a cellular scale, abundant data from computer simulations[12] suggest that restitution dynamics plays an important role in the mechanism of discordant alternans between cells. According to this theory, interacting dynamics of conduction and APD restitution determine the phase of APD alternans between neighboring cells and, in turn, the spatial organization of repolarization in the heart. However, this simple, albeit elegant, explanation for alternans may not account for the complexities in action potential dynamics associated with alternans. For example, during cellular alternans, restitution is not defined by a single curve but by two curves, depending on whether the premature stimulus follows the longer APD or the shorter APD.[13] Therefore, cellular alternans causes restitution kinetics to vary from beat to beat (i.e.,

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temporal heterogeneities of restitution). Restitution kinetics vary not only temporally but spatially between cells located in the ventricular apex and base[14] or epicardium and endocardium.[15] Importantly, these spatial heterogeneities of restitution closely correspond to the spatial orientation of discordant alternans that develops in response to an elevated heart rate,[5] suggesting a role for spatial heterogeneities of restitution in the mechanism of discordant alternans. In the present study, we used high-resolution optical mapping to track dynamic changes of alternans between cells in order to test the hypothesis that spatial and temporal heterogeneities of restitution provide a mechanism of arrhythmogenic discordant alternans.

Methods

Experimental preparation

Experiments were performed in the guinea pig model of pacing-induced TWA as described previously.[5,16] Briefly, hearts from nine male retired breeder guinea pigs were rapidly excised and perfused as Langendorff preparations under constant flow conditions. The flow rate was adjusted to maintain aortic pressure between 50 and 60 mmHg. The endocardial surface was eliminated using a cryoablation procedure to restrict propagation to the surface from which action potentials were recorded.[5,17] Hearts were stained with 100 mL of the voltage-sensitive dye di-4-ANEPPS (15 μM) by direct coronary perfusion for 10 minutes.

Beating and perfused hearts were placed in a custom-built imaging chamber. Three silver disk electrodes fixed to the chamber in positions corresponding to ECG limb leads I, II, and III were used to measure ECG signals that were filtered (0.05–1,000 Hz) and amplified (1,000×). ECG signals were recorded simultaneously with action potential maps and displayed on a digital oscilloscope to continuously monitor TWA throughout the experiment.

Optical mapping system

Action potentials were recorded simultaneously from 256 sites on the anterior surface of the guinea pig ventricle based on a tandem-lens optical mapping system described previously.[18] In this investigation, the system was used to map action potentials from a 14- × 14-mm area with high spatial (0.88 mm), temporal (1 ms), and voltage (0.5 mV) resolutions. As in previous studies, no agent to suppress contractility (e.g., diacetyl monoxime) was used.

Stimulation protocol

This study's primary goal was to investigate the role of spatial and temporal heterogeneities in restitution on development of discordant alternans. In each experiment, the epicardial surface was stimulated at 5× diastolic threshold current using a bipolar electrode placed on the basal portion of the anterolateral left ventricular surface. Concordant (i.e., spatially "in phase") cellular alternans was elicited by progressively decrementing the steady-state pacing cycle length (CL) until visible TWA was observed on the ECG. At this steady-state alternans cycle length (ALT-S1) (average cycle length 221 ± 13 ms), a single extrastimulus (S2) was applied (with no compensatory pause, i.e., S2S1 interval = S1S1 interval) every 51st beat so that S2 followed the even beat on one drive train and the odd beat on the next (Figure 1A). The even beat was defined as the beat exhibiting a prolonged APD. The cellular response to S2 was determined by recording action potentials simultaneously from 256 sites for the last three beats of the drive train and the beat following S2. The S2 was applied at coupling intervals ranging from 10 ms greater than the ALT-S1 to refractoriness (in

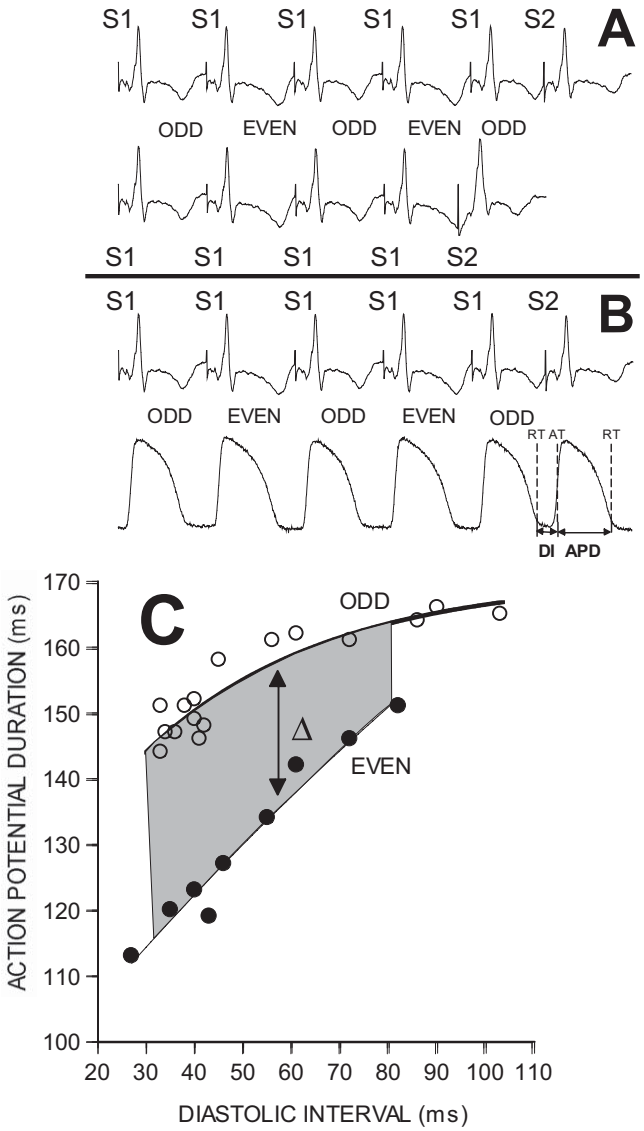

Figure 1 Alternans restitution protocol. **A:** A single premature stimulus (S2) was applied following both the even beat **(bottom)** and the odd beat **(top)** during steady-state T-wave alternans. **B:** One of 256 action potentials recorded during the protocol using optical mapping and the relationship between activation time (AT), recovery time (RT), action potential duration (APD), and diastolic interval (DI). **C:** Restitution curves for the even beat *(filled circles)* and the odd beat *(open circles)* of alternans from data generated at one ventricular site. Both curves were fit to exponentials. The *shaded region* corresponds to the area used to calculate average beat-to-beat restitution separation defined as Δ-restitution.