

# Modulated Dispersion Explains Changes in Arrhythmia Vulnerability During Premature Stimulation of the Heart

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**Background**—Previously, we have shown that a premature stimulus can significantly modulate spatial gradients of ventricular repolarization (ie, modulated dispersion), which result from heterogeneous electrophysiological properties between cells. The role modulated dispersion may play in determining electrical instability in the heart is unknown.

**Methods and Results**—To determine if premature stimulus-induced changes in repolarization are a mechanism that governs susceptibility to cardiac arrhythmias, optical action potentials were recorded simultaneously from 128 ventricular sites (1 cm<sup>2</sup>) in 8 Langendorff-perfused guinea pig hearts. After baseline pacing (S<sub>1</sub>), a single premature stimulus (S<sub>2</sub>) was introduced over a range of S<sub>1</sub>S<sub>2</sub> coupling intervals. Arrhythmia vulnerability after each premature stimulus was determined by measurement of a modified ventricular fibrillation threshold (VFT) during the T wave of each S<sub>2</sub> beat (ie, S<sub>2</sub>-VFT). As the S<sub>1</sub>S<sub>2</sub> interval was shortened to an intermediate value, spatial gradients of repolarization and vulnerability to fibrillation decreased by 51±9% (mean±SEM) and 73±45%, respectively, compared with baseline levels. As the S<sub>1</sub>S<sub>2</sub> interval was further shortened, repolarization gradients increased above baseline levels by 54±30%, which was paralleled by a corresponding increase (37±8%) in vulnerability.

**Conclusions**—These data demonstrate that modulation of repolarization gradients by a single premature stimulus significantly influences vulnerability to ventricular fibrillation. This may represent a novel mechanism for the formation of arrhythmogenic substrates during premature stimulation of the heart. (*Circulation*. 1998;98:2774-2780.)

**Key Words:** action potentials ■ arrhythmia ■ reentry ■ mapping ■ pacing

A premature impulse is traditionally viewed as a “trigger” for reentrant arrhythmias that, in the presence of a suitable arrhythmogenic “substrate,”<sup>1</sup> can provoke reentry. The essential components of the substrate, such as nonuniformities of recovery<sup>2-4</sup> and tissue anisotropy,<sup>5,6</sup> are thought to remain unchanged during a premature beat. Accordingly, the increase in arrhythmia vulnerability associated with multiple premature stimuli delivered at progressively shorter coupling intervals is thought to result from shortening (ie, similar to “peeling back”<sup>7</sup>) of refractoriness at the pacing site, allowing capture of subsequent stimuli at increasing degrees of prematurity.<sup>8</sup> Although this paradigm is based on the well-established dependence of cellular repolarization/refractoriness on stimulus prematurity,<sup>9,10</sup> it only considers what happens to repolarization at one ventricular site and does not account for spatial variations in the response of cellular repolarization to a premature stimulus. Such a response would suggest that a premature stimulus may not only serve as a trigger for arrhythmias but may also produce significant changes in dispersion of refractoriness that forms the underlying electrophysiological substrate for reentry.

There is now considerable evidence that membrane ionic processes that govern the extent of action potential duration

(APD) shortening after a premature stimulus (ie, APD restitution<sup>11</sup>) vary substantially throughout the heart.<sup>12-16</sup> In particular, we have shown previously<sup>17</sup> that systematic heterogeneities of APD restitution between cells across the epicardial surface govern, in a coupling-interval-dependent fashion, premature stimulus-induced modulation of spatial gradients of repolarization. Because spatial gradients of repolarization are closely associated with reentrant arrhythmogenesis,<sup>3,18</sup> modulation of such gradients may play an important role in the mechanisms of cardiac arrhythmias.

Therefore, an alternative hypothesis is that a premature stimulus also alters the underlying arrhythmogenic substrate by modulating spatial gradients of repolarization in a coupling-interval-dependent manner, ie, modulated-dispersion hypothesis. However, because of limitations of conventional recording techniques, dynamic changes in the spatial pattern and organization of repolarization during a premature stimulus are poorly understood. To test the modulated-dispersion hypothesis, high-resolution action potential mapping with voltage-sensitive dye was used to measure spatial gradients of cellular repolarization during a premature stimulus delivered over a broad range of coupling intervals. We found that a

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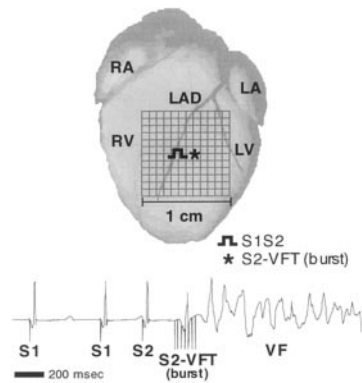
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**Figure 1.** Diagram of the mapping field (1 cm<sup>2</sup> grid) and its position relative to the intact heart. Each small box within the grid (0.08×0.08 mm) represents a single recording site. Baseline pacing (S<sub>1</sub>) and first premature stimulus (S<sub>2</sub>) were always delivered from the same site (stimulus symbol). ECG shown below depicts last 2 beats of drivetrain (S<sub>1</sub>) followed by a single premature stimulus (S<sub>2</sub>). Shown is a normal heart in which VF was induced by delivery of a burst train of stimuli (100 Hz) during ECG T wave of S<sub>2</sub> beat (S<sub>2</sub>-VFT) at a second site nearby (\*). RA indicates right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; and LAD, left anterior descending coronary artery.

premature stimulus not only serves to “trigger” arrhythmias but also importantly modulates spatial gradients of repolarization and, as a result, the electrophysiological substrate for ventricular fibrillation (VF).

## Methods

### Experimental Preparation

All experiments were performed in accordance with Public Health Service guidelines for the care and use of laboratory animals. Guinea pigs (n=8) were anesthetized (pentobarbital sodium 30 mg/kg IP) and their hearts rapidly excised and perfused as Langendorff preparations (perfusion pressure, 70 mm Hg) with oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Tyrode's solution containing (in mmol/L) NaCl 130, NaHCO<sub>3</sub> 25.0, MgSO<sub>4</sub> 1.2, KCl 4.75, dextrose 5.0, and CaCl<sub>2</sub> 1.25 (pH 7.40, 31°C to 33°C). The right atrium was excised to avoid competitive stimulation from the sinoatrial node. Hearts were stained with voltage-sensitive dye di-4-ANEPPS (10 μmol/L) by direct coronary perfusion for 10 minutes.

Beating and perfused hearts were immersed in a Tyrode's solution-filled, custom-built acrylic plastic chamber that was attached to a micromanipulator so that the mapping field could be centered over the left anterior descending coronary artery ≈6 mm below its bifurcation with the diagonal coronary artery (Figure 1). Gentle pressure was applied with a movable piston to the posterior surface of the heart to stabilize the anterior surface of the ventricle against an imaging window, which reduced motion artifacts. This design permitted the heart to contract freely during action potential recordings except for the 1-cm<sup>2</sup> area within the mapping field, while the electrophysiological side effects associated with pharmacological suppression of cardiac motion were avoided.<sup>19–22</sup> Cardiac rhythm was monitored with 3 silver disk electrodes fixed to the chamber in positions corresponding to ECG limb leads I, II, and III. The ECG signals were filtered (0.3 to 300 Hz), amplified (1000×), and displayed on a digital recorder (Windograf, Gould Inc). Although all experiments were typically completed within 1 to 2 hours, these preparations were stable and exhibited normal action potential characteristics for ≥3 hours of Langendorff perfusion.

### Experimental Protocol

The ventricular epicardial surface was stimulated at 2× diastolic threshold with Teflon-coated silver bipolar electrodes (0.1 mm diameter, interelectrode spacing of 1.0 mm). Drivetrain (S<sub>1</sub>) and the

first premature stimulus (S<sub>2</sub>) were always delivered from the same stimulus electrode by a programmable stimulator (DTU 101, Bloom Associates Ltd). Action potentials were recorded from each of 128 ventricular recording sites during the last 3 beats of a 50-beat drivetrain and during premature stimulation. Premature coupling intervals (S<sub>1</sub>S<sub>2</sub>) were decreased progressively by a minimum of 5 ms until ventricular refractoriness was reached. To ensure that our results were independent of the order of coupling intervals tested, S<sub>1</sub>S<sub>2</sub> was progressively increased from ventricular refractoriness to the baseline cycle length in several experiments.

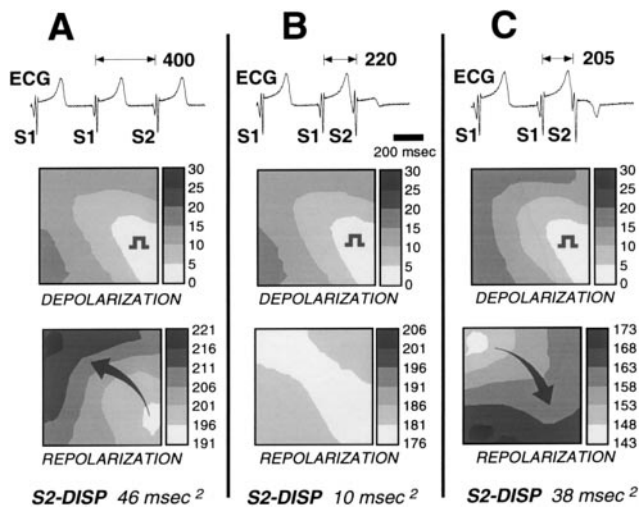
Experiments were designed to establish a relationship between coupling-interval-dependent modulation of repolarization gradients and susceptibility to VF. In all hearts, the dependence of arrhythmia vulnerability on S<sub>1</sub>S<sub>2</sub> coupling interval was measured by use of a modified VF threshold test (ie, S<sub>2</sub>-VFT). S<sub>2</sub>-VFT was used as a measure of vulnerability of the heart to VF after a single (S<sub>2</sub>) premature stimulus. The S<sub>2</sub>-VFT test stimulus was delivered by use of a Teflon-coated silver bipolar electrode (0.1 mm diameter, interelectrode spacing of 1.0 mm) connected to a second programmable stimulator (DCI-1114, Digital Cardiovascular Instruments Inc). Baseline pacing (S<sub>1</sub>S<sub>1</sub> ranging from 300 to 400 ms) and a single premature stimulus (S<sub>2</sub>) were delivered from the same site near the center of the mapping field (Figure 1). The site of S<sub>2</sub>-VFT stimulation was always positioned near (<4 mm) the S<sub>1</sub>S<sub>2</sub> stimulation site. To determine S<sub>2</sub>-VFT, a burst pulse train (100 Hz) was applied during the T wave of the S<sub>2</sub> beat (Figure 1). Because the pacing protocol required shortening of the S<sub>1</sub>S<sub>2</sub> coupling interval over a broad range, we adjusted the timing of burst stimulation to account for shortening in the QT interval of the ECG. For each coupling interval tested, QT interval was measured and the timing of the burst train adjusted to span the entire T wave of the S<sub>2</sub> beat. To verify its onset and termination relative to the T wave, a test burst train was delivered below threshold. The number of pulses that extended beyond the end of the T wave was kept constant throughout the experiment. This ensured that for every coupling interval tested, an equal number of pulses traversed the vulnerable period and, if present, the protective zone of the S<sub>2</sub> beat.<sup>23</sup> The S<sub>2</sub>-VFT burst stimulus was repeated for the same S<sub>1</sub>S<sub>2</sub> coupling interval at increasing current strengths until sustained VF occurred. S<sub>2</sub>-VFT was defined by the minimum current strength that initiated VF. After induction of fibrillation, the heart was defibrillated (0.8 J) and allowed to equilibrate at the baseline pacing rate for 3 minutes. To assure reproducibility, the S<sub>2</sub>-VFT measurement was repeated 2 to 3 times for each S<sub>1</sub>S<sub>2</sub> coupling interval tested.

### Optical Mapping System

The action potential mapping system used in the present study was described in detail elsewhere.<sup>17,19,24</sup> Briefly, to make quantitative measurements of action potential characteristics, including depolarization and repolarization, our optical mapping system was designed to record high-fidelity action potentials simultaneously from 128 sites on the intact heart. In the present study, an optical magnification of 1.8× was used, corresponding to a mapping field of 1 cm<sup>2</sup> and 0.83-mm spatial resolution between recording sites. Action potentials recorded from each photodiode element and the ECG signals were multiplexed, digitized with 12-bit precision, and sampled at 2000 Hz/channel to the hard disk of a UNIX workstation (Concurrent 5450S, Concurrent Computer Corp). The signal-to-noise ratio and dynamic range of these recordings allowed detection of voltage changes as small as 1 mV (based on a 100-mV action potential). Data acquisition software was used to control the onset and duration of recordings and display data for on-line inspection by the investigators. Data were analyzed off-line with the use of UNIX workstations (Sun Sparcstation, Sun Microsystems) connected to the data acquisition computer through a fiber optic network.

### Action Potential Analysis

In all experiments and for each S<sub>1</sub>S<sub>2</sub> coupling interval tested, the spatial patterns and gradients of depolarization, repolarization, and APD were represented as contour maps. Both depolarization time and repolarization time were measured relative to a single fiducial



**Figure 2.** Dependence of depolarization and repolarization on premature coupling interval from a representative experiment (experiment 8). ECGs (top) and contour maps of depolarization (middle) and repolarization (bottom) during a premature stimulus delivered at a coupling interval near the baseline pacing cycle length (A), at an intermediate coupling interval (B), and at a short coupling interval near the effective refractory period (C). All values shown are in milliseconds unless otherwise indicated. The sites of baseline pacing and premature stimulation (stimulus symbol) were identical for each coupling interval. Dispersion of repolarization (ie,  $S_2$ -DISP) was calculated for each  $S_1$  $S_2$  coupling interval shown.

point (ie, the stimulus) and were determined by use of previously validated computer algorithms.<sup>19,25</sup> Depolarization time was defined as the point of maximum positive derivative in the action potential upstroke ( $dV/dt_{max}$ ). Repolarization time was calculated for the  $S_2$  beat and was defined by the maximum positive curvature (maximum positive second derivative) during repolarization. APD was defined as the difference between repolarization time and depolarization time. Software was written to display action potentials and computer-assigned depolarization and repolarization times for review by the investigator. The dependence of repolarization time on  $S_1$  $S_2$  coupling interval was estimated by calculation of the mean repolarization time over all 128 mapping sites (ie,  $S_2$ -RT). To quantify spatial dispersion of repolarization caused by repolarization gradients, the variance of repolarization times over all sites was calculated for each  $S_2$  beat (ie,  $S_2$ -DISP).

For each experiment, arrhythmia vulnerability (ie,  $S_2$ -VFT), mean repolarization time (ie,  $S_2$ -RT), and dispersion of repolarization (ie,  $S_2$ -DISP) were compared at 3 predefined  $S_1$  $S_2$  coupling intervals: a coupling interval equal to or near the baseline drivetrain ( $S_1$  $S_1$ ), an intermediate coupling interval, and a short coupling interval just beyond the effective refractory period of the  $S_1$  beat. The intermediate coupling interval was defined as the coupling interval when dispersion of repolarization of the  $S_2$  beat (ie,  $S_2$ -DISP) reached a minimum. Levels of significance were determined by the Wilcoxon matched-pairs test. All values shown are mean  $\pm$  SD unless otherwise indicated.

## Results

### Gradients of Repolarization and Modulated Dispersion During a Premature Stimulus

Figure 2 illustrates a representative example of spatial gradients of depolarization and repolarization measured during a premature beat ( $S_2$ ) delivered at a coupling interval equal to the drivetrain (400 ms, Figure 2A), at an intermediate coupling interval (220 ms, Figure 2B), and at a short coupling interval near refractoriness (205 ms, Figure 2C). For an  $S_1$  $S_2$

coupling interval equal to the drivetrain (Figure 2A), the prematurely stimulated ( $S_2$ ) impulse propagated anisotropically from the site of pacing. Significant gradients of APD were present across the epicardial surface (not shown), as evidenced by gradients of repolarization that produced a spatial dispersion of repolarization times (46  $ms^2$ ). A premature stimulus introduced at an intermediate coupling interval (Figure 2B) produced no change in the pattern of depolarization compared with baseline pacing. In contrast, repolarization gradients were substantially reduced, minimizing dispersion of repolarization to 10  $ms^2$ . The significant reduction of repolarization gradients is also evident in the ECG by the diminished T-wave amplitude during the  $S_2$  beat. When a premature stimulus was introduced at a short coupling interval near refractoriness (Figure 2C), conduction slowed somewhat, but the overall pattern of depolarization was unchanged. In contrast, repolarization gradients reappeared. The gradients of repolarization increased dispersion to levels (38  $ms^2$ ) comparable to that during baseline pacing. In addition, the orientation of the repolarization gradients (arrows in Figure 2) was reversed; ie, where repolarization was latest during baseline pacing, it became earliest during the short premature coupling interval and vice versa. The reversal of repolarization gradients was also evidenced by inversion of the ECG T wave of the  $S_2$  beat relative to the drivetrain beat. The changes observed in T-wave morphology suggest that modulation of repolarization gradients was not limited to the optical mapping field but was most likely occurring throughout the heart.

The spatial dispersion of repolarization observed during baseline pacing and during premature stimulation is summarized for all experiments in the Table. The initial decrease and subsequent increase (ie, biphasic modulation) in dispersion of repolarization as the coupling interval was shortened were observed in all experiments. In general, dispersion changed more rapidly at short coupling intervals than at long coupling intervals. As the  $S_1$  $S_2$  coupling interval was shortened from baseline to an intermediate value, dispersion decreased by 27  $ms^2$ ; however, as  $S_1$  $S_2$  was further reduced from intermediate to short coupling intervals, dispersion increased by 54  $ms^2$ . With the exception of 2 experiments (experiments 7 and 8), dispersion of repolarization was always greater at short coupling intervals than at baseline. These data indicate that dispersion of repolarization was predictably and significantly modulated by simply changing the timing of a single premature stimulus.

### Vulnerability to Fibrillation

To determine vulnerability to VF in the wake of repolarization gradients induced by a premature stimulus, we initiated VF by applying a burst train of stimuli during the vulnerable period of the premature beat ( $S_2$ -VFT). Figure 3 demonstrates the initiation of VF after a premature beat delivered at a coupling interval equal to the drivetrain (400 ms, Figure 3A), at an intermediate coupling interval (210 ms, Figure 3B), and at a short coupling interval near refractoriness (185 ms, Figure 3C). The ECG and transmembrane potential recorded from 1 ventricular site are shown for each coupling interval. For an  $S_1$  $S_2$  coupling interval equal to the drivetrain (Figure



## Influence of Premature Coupling Interval on Dispersion of Repolarization

Experiment No.	Coupling Interval, ms			S <sub>2</sub> -DISP, ms <sup>2</sup>		
	BASE	INTER	SHORT	BASE	INTER	SHORT
1	300	255	230	103	78	110
2	300	245	220	43	16	136
3	300	250	220	110	36	121
4	300	270	230	47	23	108
5	400	250	220	18	9	24
6	350	230	195	64	59	123
7	400	230	215	24	7	14
8	400	220	205	46	10	48
Mean±SD	344±50	244±16	217±12	57±34	30±26	84±50
Δ		-100	-27		-27	54

Summary of experimental data demonstrating the effect of premature stimulation coupling interval on dispersion (variance) of repolarization (S<sub>2</sub>-DISP). Shown are results for baseline stimulation (BASE), premature stimulation at an intermediate coupling interval (INTER), and premature stimulation at a short coupling interval near refractoriness (SHORT). The change in coupling interval and S<sub>2</sub>-DISP as S<sub>1</sub>S<sub>2</sub> was decreased from BASE to INTER and INTER to SHORT is shown across the bottom (Δ). The intermediate coupling interval (INTER) was defined by the S<sub>1</sub>S<sub>2</sub> coupling interval that resulted in minimal dispersion.

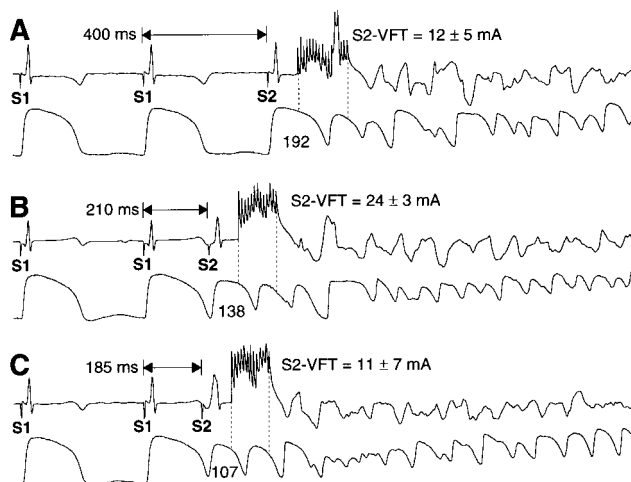
3A), APD of the S<sub>2</sub> beat (192 ms) was unchanged compared with the drivetrain, and the average S<sub>2</sub>-VFT was 12±5 mA. When S<sub>1</sub>S<sub>2</sub> coupling interval was shortened to an intermediate value of 210 ms (Figure 3B), APD of the S<sub>2</sub> beat decreased significantly (138 ms) compared with the drivetrain; however, the average S<sub>2</sub>-VFT increased to 24±3 mA, indicating a paradoxical 50% decrease in arrhythmia vulnerability. As the S<sub>1</sub>S<sub>2</sub> coupling interval was further shortened (185 ms), APD continued to decrease (107 ms), and S<sub>2</sub>-VFT (11±7 mA) decreased to levels observed during drivetrain pacing (Figure 3C). Therefore, despite the fact that APD shortened

monotonically as the S<sub>1</sub>S<sub>2</sub> coupling interval was shortened, arrhythmia vulnerability first decreased markedly (ie, S<sub>2</sub>-VFT increased) with shortening of the S<sub>1</sub>S<sub>2</sub> coupling interval and then increased only at very short S<sub>1</sub>S<sub>2</sub> intervals.

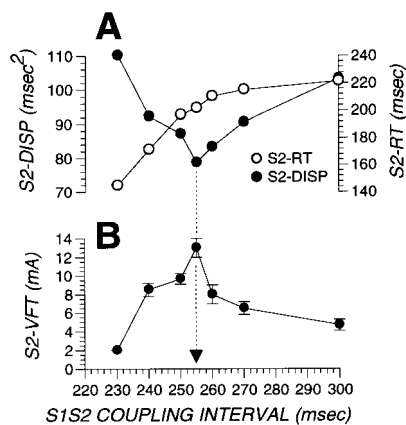
Because optically recorded action potentials are immune to stimulus artifacts, it was possible to clearly identify the beats captured by the burst train. The transmembrane potentials shown in Figure 3 demonstrate that for each coupling interval tested, only 1 beat was captured by the burst train, as evidenced by the initiation of only a single action potential upstroke between the dashed lines. Similar results were observed for all experiments. This indicates that in the wake of S<sub>2</sub> repolarization, only a single beat was captured by the burst train. Hence, modulation of the electrophysiological substrate (ie, repolarization gradients) by the S<sub>2</sub> beat directly influenced the response of the beat subsequently captured by the burst train that initiated VF.

## Modulated Dispersion and Vulnerability to Fibrillation

To quantitatively determine the relationship between S<sub>1</sub>S<sub>2</sub> coupling interval and repolarization properties of the ventricle, mean repolarization time (ie, S<sub>2</sub>-RT) and dispersion of repolarization time (ie, S<sub>2</sub>-DISP) were calculated over a broad range of S<sub>1</sub>S<sub>2</sub> coupling intervals. Figure 4A shows the mean and dispersion of repolarization times generated during each prematurely stimulated beat from a representative experiment. S<sub>2</sub>-RT decreased monotonically from 221 to 145 ms as the S<sub>1</sub>S<sub>2</sub> coupling interval was shortened from 300 to 230 ms. These changes were attributed to coupling-interval-dependent changes in APD, which were most marked at short S<sub>1</sub>S<sub>2</sub> coupling intervals, as predicted from restitution properties of cardiac myocytes.<sup>9</sup> In contrast, S<sub>2</sub>-DISP was modulated in a biphasic fashion as coupling interval was shortened. For S<sub>1</sub>S<sub>2</sub> coupling intervals near the baseline pacing rate, dispersion of repolarization was relatively high; however, as S<sub>1</sub>S<sub>2</sub> coupling interval was shortened, dispersion of repolarization decreased



**Figure 3.** Measuring vulnerability to VF (ie, S<sub>2</sub>-VFT) by burst stimulation (100 Hz) during vulnerable period of first premature (S<sub>2</sub>) beat. Shown are ECG (top) and 1 optically recorded ventricular transmembrane potential (bottom) measured during initiation of VF at 3 different S<sub>1</sub>S<sub>2</sub> coupling intervals (A, 400 ms; B, 210 ms; C, 185 ms). For each coupling interval, the last 2 beats of drivetrain pacing (S<sub>1</sub>) and the first premature stimulus (S<sub>2</sub>) followed by the onset of VF are shown. S<sub>2</sub>-VFT is the average over 2 to 3 repeated initiations. In each panel, APD of the S<sub>2</sub> beat is shown under each S<sub>2</sub> action potential, and dashed lines indicate time interval when burst train was delivered.

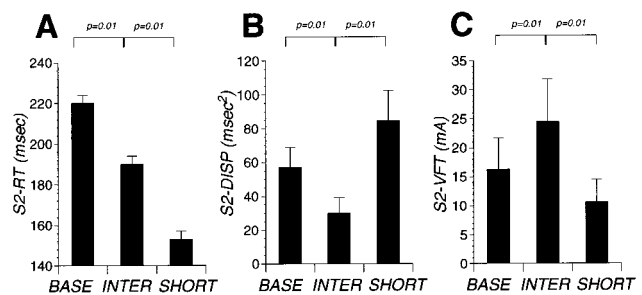


**Figure 4.** A, Dependence of mean repolarization ( $S_2$ -RT, open circles) and dispersion of repolarization ( $S_2$ -DISP, filled circles) on  $S_1S_2$  coupling interval from a representative experiment. B, Dependence of arrhythmia vulnerability ( $S_2$ -VFT) on premature coupling interval from same representative experiment. Dispersion of repolarization (A, filled circles) and vulnerability to fibrillation (B) were modulated in a similar biphasic fashion, with minimum vulnerability (ie, maximum  $S_2$ -VFT) and minimum dispersion occurring at the same  $S_1S_2$  coupling interval (255 ms, dashed arrow).

until a critical coupling interval was reached (255 ms; dashed arrow in Figure 4). With additional shortening of the  $S_1S_2$  coupling interval, dispersion of repolarization rose sharply to a level slightly higher than that measured during baseline pacing.

To examine the relationship between repolarization gradients and arrhythmia vulnerability, the VF threshold after the premature beat (ie,  $S_2$ -VFT) was measured for all  $S_1S_2$  coupling intervals tested. Figure 4B shows  $S_2$ -VFT measured from the same experiment as in Figure 4A. It is evident that vulnerability was modulated in a biphasic fashion in parallel with dispersion of repolarization (filled circles, Figure 4A). As the  $S_1S_2$  coupling interval was shortened to a critical value (dashed arrow),  $S_2$ -VFT increased (ie, vulnerability decreased). With additional shortening of  $S_1S_2$ ,  $S_2$ -VFT decreased (ie, vulnerability increased) to levels below those present at baseline pacing. Therefore, during premature stimulation of the heart, biphasic changes in vulnerability to fibrillation coincided exactly with coupling-interval-dependent changes in dispersion of repolarization but not with changes in mean repolarization time.

The results from all 8 experiments are summarized in Figure 5, which shows mean repolarization time (Figure 5A), dispersion of repolarization (Figure 5B), and arrhythmia vulnerability (Figure 5C) after a single premature stimulus. Values are shown for coupling intervals near baseline pacing rates ( $344 \pm 50$  ms), intermediate coupling intervals ( $244 \pm 16$  ms), and short coupling intervals just beyond refractoriness of the  $S_1$  beat ( $217 \pm 12$  ms). In all experiments, mean repolarization time decreased significantly in a monotonic fashion as the  $S_1S_2$  coupling interval was progressively shortened (Figure 5A). However, dispersion of repolarization was modulated in a biphasic fashion, as evidenced by a significant reduction as  $S_1S_2$  coupling interval was shortened and then a sharp increase in dispersion as  $S_1S_2$  approached refractoriness (Figure 5B). In general, there was a trend for dispersion of



**Figure 5.** Summary data for 8 experiments showing mean repolarization ( $S_2$ -RT, A), dispersion of repolarization ( $S_2$ -DISP, B), and vulnerability to fibrillation ( $S_2$ -VFT, C) after premature stimulation for coupling intervals near baseline pacing rates (BASE), for intermediate coupling intervals (INTER), and for short coupling intervals near refractoriness (SHORT). All values shown are mean  $\pm$  SEM. Mean repolarization time decreased monotonically, whereas dispersion of repolarization and arrhythmia vulnerability were modulated analogously in a biphasic fashion.

repolarization at short  $S_1S_2$  coupling intervals to be greater than that at baseline pacing rates. Arrhythmia vulnerability was also modulated in a biphasic fashion as the  $S_1S_2$  coupling interval was shortened (Figure 5C). At short  $S_1S_2$  coupling intervals, arrhythmia vulnerability was always greater than that at baseline pacing rates. The biphasic coupling-interval dependence exhibited by  $S_2$ -VFT supports a mechanistic relationship between arrhythmia vulnerability and modulated dispersion, more so than changes in mean refractoriness.

## Discussion

In this study, we report a potentially important mechanism of initiation of VF based on the direct effect of a premature stimulus on the electrophysiological substrate for reentry. We found that vulnerability to fibrillation was modulated in a coupling-interval-dependent manner by pacing-induced modulation of repolarization gradients. Such modulation of repolarization gradients explained a paradoxical reduction of arrhythmia vulnerability after premature stimulation at intermediate coupling intervals, followed by a sharp increase in vulnerability at coupling intervals approaching refractoriness. Our results suggest that the “trigger” and the “substrate” for reentry are not independent, and the manner in which the electrophysiological substrate is modulated by a premature stimulus can strongly influence the state of cardiac electrical instability.

Previously, we<sup>17</sup> have shown that a premature stimulus can systematically modulate spatial gradients of APD in a coupling-interval-dependent manner, which was explained on the basis of heterogeneities of APD restitution kinetics between ventricular cells. Although repolarization was dependent on the combined influence of APD and propagation, our data indicate that modulation of repolarization gradients was primarily due to coupling-interval-dependent changes in APD. For example, as the  $S_1S_2$  coupling interval was shortened to an intermediate value, the pattern of depolarization was unchanged (Figure 2), whereas gradients of repolarization were essentially eradicated because of a significant reduction of APD gradients. As the  $S_1S_2$  coupling interval was further shortened, conduction slowed slightly and APD gradients reappeared, both of which combined to increase the

magnitude (ie, dispersion) of repolarization gradients. In general, dispersion of repolarization at the shortest  $S_1S_2$  coupling intervals was greater than at baseline pacing. It is possible that slow conduction at short  $S_1S_2$  coupling intervals preferentially delayed repolarization away from the site of stimulation and further enhanced dispersion of repolarization. In any case, biphasic modulation of APD gradients played a dominant role in determining repolarization gradients created by a premature stimulus.

In the present study, we found that stimulus-induced changes in repolarization gradients directly influenced vulnerability to fibrillation. As the  $S_1S_2$  coupling interval was shortened to an intermediate value, dispersion of repolarization and arrhythmia vulnerability decreased in parallel (Figure 5). From a teleological standpoint, it is possible that such attenuation of repolarization gradients observed at intermediate premature coupling intervals serves as a protective mechanism against arrhythmias during premature stimuli. On the other hand, in some pathological conditions, this mechanism may be lost, which can explain why under such circumstances, less aggressive pacing protocols (ie, intermediate  $S_1S_2$  coupling intervals) are capable of inducing arrhythmias.<sup>26</sup> Additional shortening of the  $S_1S_2$  coupling interval to a value just longer than the effective refractory period markedly accentuated repolarization gradients, which in turn produced conditions necessary for the development of fibrillation. For all experiments, arrhythmia vulnerability was higher (ie, smaller VFT) at the shortest  $S_1S_2$  coupling intervals than with baseline pacing (Figure 5C). This may be explained by conduction slowing and reduced refractoriness at very short  $S_1S_2$  coupling intervals, both of which (in addition to increased dispersion of repolarization) are expected to promote reentrant excitation. The rapid increase in vulnerability at very short coupling intervals is consistent with the common observation that the initiation of VF in normal hearts typically required multiple closely coupled premature stimuli.<sup>26</sup>

Relatively little attention has been given to how a premature stimulus alters the electrophysiological substrate for reentry in general and gradients of repolarization in particular throughout the heart. It is generally assumed that the effect of shortening the premature stimulus coupling interval is to increase the likelihood of inducing reentry by shortening refractoriness (ie, wavelength). However, this notion is somewhat limited because it does not account for spatial heterogeneities in cellular responses to a premature stimulus and, thus, changes in the spatial pattern of refractoriness during premature stimulation. Accordingly, we hypothesized that a premature stimulus influenced arrhythmia vulnerability by altering gradients of refractoriness. Early studies supported this concept by showing that tightly coupled premature stimuli increased dispersion of repolarization.<sup>27</sup> However, those studies were limited in their ability to resolve spatial gradients of repolarization, and dispersion of repolarization was only measured for  $S_1S_2$  coupling intervals near the effective refractory period. This may explain why the paradoxical decrease in arrhythmia vulnerability and repolarization gradients we observed as the coupling interval was initially shortened may have been unrecognized previously.

The growing awareness of heterogeneities throughout the heart (ie, from epicardium to endocardium<sup>14</sup>) raises the possibility that in the intact heart, repolarization gradients are modulated across deeper subepicardial layers as well as across the epicardial surface. We made no attempt to account for transmural heterogeneities of cellular repolarization. However, studies from limited transmural recording sites across the ventricular wall of canine hearts<sup>16</sup> have revealed mechanisms of repolarization similar to those we have observed.<sup>17</sup> Therefore, the principles set forth in the present study may apply to other situations in which a heterogeneity of membrane repolarization properties exists.

Modulation of the electrophysiological substrate by a premature stimulus in a coupling-interval-dependent manner (ie, modulated dispersion), as demonstrated in the present report, may have important implications for the mechanism of arrhythmias initiated by premature stimuli. In patients with ischemic heart disease, tachycardia is preceded by pairs or multiple ventricular ectopic beats in 55% of all spontaneous events.<sup>28</sup> Moreover, during programmed stimulation, the likelihood of inducing reentry increased as the number of extrastimuli increased.<sup>29,30</sup> It is possible that a premature stimulus preconditioned the electrophysiological substrate and, accordingly, altered the likelihood of inducing reentry by a second ( $S_3$ ) and possibly a third ( $S_4$ ) premature stimulus. The results of the present study also suggested that in some circumstances, suitable conditions for reentry may not exist during baseline pacing but can form dynamically during premature stimulation of the heart.

Using the  $S_2$ -VFT test, we were able to quantify arrhythmia vulnerability and demonstrate a significant correlation with repolarization gradients induced by a single premature stimulus (Figure 5). On the basis of these data, it is likely that the presence or absence of steep repolarization gradients directly influenced the initiation of VF. However, even though the VFT test is an established experimental technique that has been used extensively to quantify arrhythmia vulnerability,<sup>18,31,32</sup> the precise mechanism of arrhythmia initiation is not completely understood. For example, it is possible that  $>1$  stimuli in the burst train captured the tissue (ie,  $S_3$  and  $S_4$ ) before the initiation of fibrillation. However, the optically recorded transmembrane potentials confirmed that for all coupling intervals tested, only 1 beat was captured by the burst train before the onset of fibrillation (Figure 3). It is also possible that before capture, stimuli in the burst train altered transmembrane potential. Strong, shock-strength stimuli have been associated with virtual electrode effects<sup>33</sup> and graded responses,<sup>34</sup> both of which can directly influence arrhythmia initiation independently of repolarization gradients. However, we did not observe any significant change in transmembrane potential during the burst train before capture. This is not surprising because the average  $S_2$ -VFT ( $17 \pm 7$  mA, 1 ms in duration) in the present study was relatively small compared with shock-strength impulses. Most likely, modulation of repolarization gradients that we observed in the present study directly influenced the occurrence of unidirectional block.<sup>35</sup> However, additional studies are required to determine the precise mechanisms by which modulation of repolarization

gradients alters the electrophysiological requirements for reentry.

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