Demonstration of the Proarrhythmic Preconditioning of Single Premature Extrastimuli by Use of the Magnitude, Phase, and Distribution of Repolarization Alternans

Sanjiv M. Narayan, MB, CHB, MD, MS, MRCP; Bruce D. Lindsay, MD; Joseph M. Smith, MD, PhD

Background—We hypothesized that single premature extrastimuli (S2) insufficient to induce reentry produce proarrhythmic effects (proarrhythmic preconditioning) that are measurable by use of the magnitude, phase, and temporal distribution of repolarization alternans (RPA; alternate-beat fluctuations in ECG repolarization).

Methods and Results—Before programmed electrical stimulation (PES), surface ECG leads I, aVF, and V1 were recorded in 30 patients during simultaneous atrial and ventricular pacing at 500 ms with S2 coupling intervals (CIs) decreasing from 400 to 240 ms in 20-ms steps. We determined RPA magnitude (Valt) as the 0.5-cycle/beat peak after spectral decomposition of consecutive STU intervals over 64 beats immediately preceding and following each S2, RPA phase reversals as discontinuities in the even/odd phase of STU alternation, and RPA distribution as the time point of median RPA magnitude within repolarization. Eighteen patients were induced into ventricular tachycardia (VT), whereas 12 were not. Extrastimuli dynamically modulated each characteristic of RPA. S2 augmented Valt in inducible (8.2 ± 2.3 versus 6.2 ± 1.6 μV; P = 0.003) but not noninducible patients. S2 reversed RPA phase more in inducible than in noninducible patients (56.7% versus 45.3%; P = 0.02 by χ2), particularly when CI was <300 ms (66.3% versus 46.5%; P = 0.006). Finally, S2 redistributed RPA significantly later within repolarization in inducible patients. Each effect was more marked for CI ≤300 ms.

Conclusions—A single S2 increases RPA magnitude, reverses its phase, and redistributes it later in repolarization in patients with the substrates for VT. These effects become more pronounced with shorter coupling intervals. These results suggest that it is possible to track the dynamic proarrhythmic preconditioning of single premature depolarizations. (Circulation. 1999;100:1887-1893.)

Key Words: tachyarrhythmias ■ pacing ■ computers ■ waves ■ depolarizing ■ death, sudden

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From the Division of Cardiology/Electrophysiology, Washington University School of Medicine, St. Louis, Mo.


Correspondence to Dr Sanjiv M. Narayan, Division of Cardiology/Electrophysiology, Campus Box 8086, Washington University School of Medicine, 660 S Euclid Ave, St. Louis, MO 63110. E-mail snarayan@im.wustl.edu

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Sudden cardiac death resulting from ventricular tachycardia (VT) or fibrillation (VF)1 occurs with an annual incidence of 1 to 2/1000 in the United States.2 However, the manner in which “experiments of nature” such as premature ventricular depolarizations dynamically modulate electrical instability to facilitate these arrhythmias remains unclear and unmeasured. Although a series of extrastimuli may induce reentrant arrhythmias during programmed electrical stimulation (PES) of the ventricle, the proarrhythmic effects of single premature depolarizations that do not induce echo beats or initiate reentry remain poorly defined and unmeasured.

We set out to determine whether a single ventricular premature depolarization may alter repolarization dynamics and increase the propensity for ventricular arrhythmias in susceptible individuals (proarrhythmic preconditioning) using repolarization alternans (RPA). RPA measures microvolt-level fluctuations in the ECG STU segment occurring on an alternate-beat basis, reflects spatial3 and temporal4–6 dispersion of repolarization, and has been linked with ventricular arrhythmias experimentally7,8 and clinically.7,9 However, work to date has focused on the static relationship between clinical arrhythmic outcome and RPA magnitude at steady-state heart rates.10,11

Three lines of experimental evidence suggest that distinct characteristics of RPA may reflect dynamic changes in myocardial electrical instability. First, ventricular arrhythmias become more likely with increasing magnitude of action potential duration (APD) alternans (in feline myocytes12 and intact guinea pig9 and canine13 hearts) and RPA (in hypothermic7 and ischemic8,14 canine hearts). Second, the phase of RPA is not static; phase reversal in the alternation of isolated myocytes12 and opposite phase of alternation between adjacent regions of Langendorff-perfused guinea pig heart15
portend ventricular arrhythmias. Third, we recently reported that RPA has a nonuniform temporal distribution within a beat. RPA late in repolarization better reflects reentrant substrates than early RPA, and furthermore, RPA temporally redistributes later with heart rate acceleration.

We therefore hypothesized that the proarrhythmic preconditioning of a single premature depolarization may be reflected by an increased magnitude, reversed phase, and later redistribution of RPA within the STU segment. We further hypothesized that such dynamics should become more marked with increasing stimulus prematurity and in individuals with demonstrable substrates for reentrant arrhythmias. We tested these hypotheses in patients undergoing PES.

Methods

Patient Recruitment and Clinical ECG Collection

Studies were approved by the Human Subjects Committee of the Washington University School of Medicine, and all subjects provided written informed consent. We recruited 30 patients referred for PES for the evaluation of unexplained syncope or VT. A subset of these patients has been studied previously. Patients were prepared for PES in the standard fashion, and antiarrhythmic medications were withheld for ≥2 days. After light sedation with intravenous midazolam, standard 6F quadripolar catheters were positioned in the right atrium and right ventricle via transvenous sheaths as part of the prescribed PES and used for simultaneous atrial and ventricular pacing.

To provide a uniform heart rate between patients, baseline pacing was applied at a cycle length of 600 ms for 2 to 5 minutes. Extrastimulus experiments then commenced. Ninety-nine beats were delivered at a cycle length of 500 ms, followed by 1 premature extrastimulus (S₂) at varying coupling intervals (CIs), then a 500-ms pause (Figure 1). The cycle was repeated with the CI shortening from 400 to 240 ms, then a 500-ms pause. Sequence then repeats at progressively shorter CIs. CL indicates cycle length.

Figure 1. Extrastimulus (S₂) protocol. Each sequence consists of 100 beats. Ninety-nine beats are presented 500 ms apart, 1 at S₂ CI (where 400≤CI≤240 ms), then a 500-ms pause. Sequence then repeats at progressively shorter S₂ CIs. CL indicates cycle length.

Spectral Computation of RPA

RPA was analyzed with interactive graphic software written by the authors in Labview (National Instruments). For each ECG lead, 64 contiguous-beat sequences avoiding ectopic or fusion beats were selected (1) before S₂ (ending with the beat preceding S₂) and (2) after S₂ (starting with and including S₂) (Figure 1). Beats were baseline corrected, then aligned to their maximal normalized QRS dot-product with a template beat, and then these 64 aligned beats were used to calculate a mean QRS complex that served as the template for a second alignment.

RPA was computed on arrays of aligned beats from each ECG lead by use of multidimensional spectral analysis over the STU segment, which was identified by an investigator who was blinded to clinical data (Figure 2). STU series were represented as 2D repolarization matrices R (n, s), where n indicates the sequence beat (0≤n≤63) and s the millisecond time sample within STU. A fast Fourier transform was used to compute power spectra (power = voltage²) across all beats n at each successive time sample s (along each vertical arrow in Figure 2, left).

Power spectra were summed into a composite in which the magnitude at 0.5 cycle/beat indicates raw alternans, $\Sigma T$ (in $\mu V$) (Figure 2a). The dimensionless T-wave alternans ratio (TWAR) represents the difference between alternans and nonalternating periodicity (spectral noise mean, $\mu_{noise}$, prospectively defined as the 10 spectral point bandwidth, 0.33 to 0.48 cycle/beat), measured in units of the SD of noise ($\sigma_{noise}$) (all in $\mu V$):

$$\text{TWAR} = \frac{\sum T - \mu_{noise}}{\sigma_{noise}}$$

where TWAR > 0 indicates RPA detectable above noise and TWAR ≥ 3 may predict ventricular arrhythmias. The mean absolute voltage difference of alternation was also estimated (Figure 2, left):

$$V_{alt} = \sqrt{\frac{\sum T - \mu_{noise}}{\text{IT duration}}}$$

RPA magnitudes were analyzed for each ECG lead and combined into the resultant vector by the Pythagorean theorem. Vector magnitudes were subsequently analyzed because of the spatial nonuniformity of RPA between leads.

Determining the Phase of RPA

RPA phase was determined relative to the position of S₂ in each 64-beat sequence. The voltage alternation of 1 STU time sample is shown in Figure 3 (top). A premature S₂ may leave the phase either unaltered (Figure 3a) or reversed (Figure 3b) in subsequent beats. Phase reversal was therefore defined by interrupted RPA oscillation (Figure 3b) in post-S₂ compared with pre-S₂ sequences.
in ≥1 surface ECG lead, providing that TWAR was >0 in both sequences in that lead.

Determining the Temporal Distribution of RPA Within Repolarization
The temporal distribution of RPA within repolarization was visualized in pre-S2 and post-S2 sequences as described previously. Briefly, RPA was reconstructed in the time domain to represent RPA magnitude for each STU time sample (Figure 2b). We used the time point of median RPA magnitude (the abscissa of the center-of-area), $x$, normalized to STU duration, $d$, to indicate RPA distribution as $T=x/d (0<T<1)$. Figure 2b illustrates centrally distributed RPA ($T/0.5$); earlier or later distributions would result in $T<0.5$ and $T>0.5$, respectively.

PES Protocol and Outcome Comparisons
PES was performed in standard fashion by pacing at the right ventricular apex. A train of 8 S1 stimuli were followed progressively with single (S2,S3), double (S2S3), or triple (S2S3S4) premature extrastimuli expected to cause inducible VT. In pre-S2 sequences, inducible patients had mean RPA magnitude higher in patients with than in those without VT. In pre-S2 sequences, inducible patients had mean RPA magnitude higher in patients with than in those without VT. Inducible patients, 3 presented with VT and the remainder with VF, and single extrastimuli did not induce echo beats or VT in any of them.

Table 1. Clinical Demographics of Study Patients

<table>
<thead>
<tr>
<th></th>
<th>VT Inducible (n=18)</th>
<th>Noninducible (n=12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n</td>
<td>15/3</td>
<td>11/1</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.1±8.5</td>
<td>64.3±10.3</td>
<td>0.3</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>33.3±12.8</td>
<td>41.1±20.4</td>
<td>NS</td>
</tr>
<tr>
<td>Prior MI, n</td>
<td>12</td>
<td>6</td>
<td>NS</td>
</tr>
</tbody>
</table>

LVEF indicates left ventricular ejection fraction; and MI, myocardial infarction.

Effect of S2 on RPA Magnitude
In each patient group, TWAR and $V_{alt}$ were higher in post-S2 than pre-S2 sequences for pooled CIs (computing the unweighted arithmetic mean of data after all CIs). However, the magnitude of post-S2 RPA augmentation was significant only in inducible patients ($V_{alt}$ increased from 6.15±1.57 to 8.22±2.30 μV for pooled CIs; $P=0.003$) and not in noninducible patients ($V_{alt}=3.98±1.65$ versus 3.94±2.18 μV; $P=NS$).

Figure 4 (top) shows that TWAR augmentation was especially marked after S2 with a short CI (≤300 ms) in inducible but not in noninducible patients, further widening the statistical difference between patient groups (pre-S2 $P=0.01$, post-S2 $P<0.005$). Conversely, longer S2 CIs (≥320 ms) augmented TWAR to a lesser extent and affected both groups similarly (Figure 4, bottom). Similar results were found for $V_{alt}$.

RPA Phase Modulation by S2
Phase reversal was significantly more common in inducible patients (and followed 56.7% or 127 of 224 presentations) than in noninducible patients (45.3% or 81 of 179 presentations; $P=0.02$ by $\chi^2$ for pooled CIs (Table 2). The effect of CI was again bimodal, with short (≤300 ms) but not longer (≥320 ms) CIs causing significantly more phase reversals in inducible than in noninducible patients ($P=0.006$; Table 2 and Figure 5). Only sequences lacking ectopy and other spurious ECG events were analyzed. Figure 5 shows that the proportion of S2 presentations causing phase reversal in patients with VT increased with progressive S2 prematurity, reaching 83.3% after the shortest CI (240 ms). This trend was not observed in noninducible patients. Importantly, there was a trend for inducible patients who showed RPA phase reversal after S2 CI 240 ms to be more readily inducible on subsequent PES (requiring a mean of 1.6 extrastimuli) than those without phase reversals (2.3 extrastimuli, $P=0.10$ by t test).

RPA Temporal Distribution After S2
RPA was distributed later within repolarization for inducible than for noninducible patients (Figure 6). In all patients, extrastimuli redistributed RPA later within repolarization for
short CIs (≤300 ms) but not CIs ≥320 ms. Furthermore, inducible patients experienced a greater redistribution that further widened the statistical difference between groups (pre-S2, T = 0.574 versus 0.524 [P < 0.02] and post-S2, T = 0.616 versus 0.562 [P < 0.005] [r test]).

When individual S2 CI was considered, the magnitude of RPA redistribution in inducible patients was significant after S2 CIs of 380 (P = 0.016), 320 (P = 0.011), 300 (P = 0.049), 280 (P = 0.014), and 240 ms (P = 0.049) (Bonferroni correction applied).

**TABLE 2. Proportion of Extrastimulus Presentations Causing RPA Phase Reversal**

<table>
<thead>
<tr>
<th>CI, ms</th>
<th>% VT-Inducible Reversal</th>
<th>% VT-Inducible No Reversal</th>
<th>% Noninducible Reversal</th>
<th>% Noninducible No Reversal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤320</td>
<td>48.7</td>
<td>51.2</td>
<td>44.1</td>
<td>55.9</td>
<td>0.494</td>
</tr>
<tr>
<td>≤300</td>
<td>66.3</td>
<td>33.6</td>
<td>46.5</td>
<td>53.5</td>
<td>0.006</td>
</tr>
<tr>
<td>All (pooled)</td>
<td>56.7</td>
<td>43.3</td>
<td>45.3</td>
<td>54.7</td>
<td>0.022</td>
</tr>
</tbody>
</table>

**Discussion**

This study is the first to demonstrate that single premature ventricular depolarizations that do not induce VT may still produce measurable repolarization effects that reflect an enhanced propensity for ventricular arrhythmias (proarrhythmic preconditioning). Single extrastimuli augment the magnitude, reverse the phase, and cause a later distribution of RPA in inducible patients. These effects become more pronounced with increasing stimulus prematurity (CI ≤300 ms), and phase reversal, in particular, correlates with the subsequent ease of induction of VT. These repolarization dynamics were not observed in patients without inducible VT.

**Pathophysiology Underlying RPA**

RPA may arise through 2 distinct mechanisms. In the first, intrinsic dispersion of refractoriness prevents the complete depolarization of myocytes with the longest recovery times (or alters their phase 2 action potential morphology).
allowing depolarization only on alternate cycles. In the second hypothesis, discordance in the generation of myocardial action potentials causes a secondary dispersion of repolarization. Extracellular recordings and voltage-sensitive dye data suggest that APD variability is primary. The dispersion of recovery in either case produces ephemeral conduction barriers that predispose to demonstrable wavefront fractionation and reentrant arrhythmias.

$S_2$ Augment RPA Magnitude

This report is the first to document the effects of premature extrastimuli on clinical RPA and their differential effects in subjects with and without VT. RPA magnitude in inducible patients was augmented by extrastimuli (Figure 4), consistent with studies of APD in nonischemic and ischemic dogs. These effects were bimodal with respect to CI, and RPA was augmented to a greater extent after CIs $\leq 300$ ms than after CIs $\geq 320$ ms (Figure 4). In noninducible patients, the small magnitude of RPA and its relative insensitivity to extrastimuli and heart rate may reflect different underlying substrates.

Mechanistically, extrastimuli may augment RPA magnitude either by modulating repolarization dispersion or by altering depolarization with secondary repolarization effects. Dispersion of repolarization has been shown to follow single and double extrastimuli in several preparations and may represent the mechanism by which long-short extrastimulus sequences induce reentry. Although the depolarization sequence may be altered by extrastimuli (for example, when CI differs from the paced cycle length by $\geq 250$ ms), the primary contribution of this mechanism to RPA requires additional study.

Extrastimuli Reverse the Alternating Phase of RPA

This is the first study of RPA phase and its modulation by extrastimuli in humans, and its results are consistent with observations of alternans phase reversal and discordance in experimental preparations. Post-$S_2$ RPA phase reversals were significantly more common in patients with than without the substrates for reentry and increased in frequency with $S_2$ prematurity (Table 2 and Figure 5).

RPA on the surface ECG likely represents a weighted integral of alternans in competing myocyte subpopulations,
each having distinct magnitude and phase. Phase reversal after appropriately timed extrastimuli may therefore reflect an altered predominance of \( \pm 1 \) subpopulation or direct reversal (“resetting”) of myocyte APD alternans. The range of CIs found to cause RPA phase reversal in the present study agrees with studies in feline myocytes\(^{12} \) and canine ventricles\(^{1} \) and, in general, may be a function of cycle length, the CI-to-cycle length relationship, and stimulus amplitude (in canine myocytes\(^{10} \)). Our finding that patients with phase reversal more readily experienced ventricular arrhythmias agrees with results of isolated myocyte studies\(^{12} \) and may represent the onset of discordant alternans: the spatial juxtaposition of subpopulations alternating with opposite phase. In cellular and tissue-level studies, discordance may lead to unidirectional conduction block\(^{15,22} \) and set the stage for reentry. Furthermore, spontaneous discordant APD alternans has been noted in quinidine-intoxicated dogs with VT/VF\(^{30} \) and ischemic canine ventricle preceding the onset of ventricular conduction block\(^{15,22} \) and ischaemic ventricular arrhythmias.\(^{3,31} \) Notably, RPA magnitude and phase may be synergistic. Greater alternans magnitudes are associated with phase reversal of APD alternans at less-premature CIs in feline myocytes\(^{12} \) and facilitate the onset of discordant ST-T alternans in dogs.\(^{31} \)

A full understanding of the relationship between RPA phase lability and dynamic arrhythmia initiation requires additional study. Although RPA phase reversal was associated with a reader-induced VT, our goal was to study the effects of stimuli insufficient to induce reentry, and we did not examine RPA phase immediately preceding VT. This may explain why even the most premature S\(_2\) produced a submaximal (84.3%) incidence of phase reversals. However, these results hint at the exciting future possibility of dynamically tailoring device or pharmacological therapy in response to measured arrhythmic susceptibility.

### RPA Redistributes Later Within Repolarization After Extrastimuli

Extrastimuli caused RPA to redistribute later within repolarization in inducible patients. The extent of temporal redistribution was again bimodal for CI and reached significance for S\(_2\) CI \( \leq \) 300 ms (Figure 6). Evidence increasingly supports the notion that the distal T wave reflects a potentially arrhythmogenic transmural gradient of repolarization in normal\(^{12} \) and long-QT syndrome physiology.\(^{33,34} \) This is reflected in the T-wave peak-to-offset interval,\(^{35} \) by dispersion of the late QT interval in Langendorff-perfused rabbit heart,\(^{35} \) and by late RPA in humans.\(^{8} \) Speculatively, a single extrastimulus may therefore create temporal variability in the trailing edge of repolarization, enabling an advancing waveform of activation to encounter inhomogeneously refractory myocardium and predisposing to waveform fractionation and reentry. This hypothesis requires additional study.

### Study Limitations

We paced the atria and ventricles simultaneously to minimize R-R (and hence repolarization) variability and to allow analysis of late repolarization unobscured by superimposed atrial activity. This method differs from other clinical studies\(^{10,11} \) and may preclude a direct comparison of RPA magnitude between studies.

As expected, there was a trend for inducible patients to have worse cardiac systolic function and a higher incidence of previous myocardial infarction than noninducible patients (Table 1). Although 1 measure of RPA has been found to be independent of the presence of structural heart disease,\(^{9,10} \) data do suggest that myocyte slippage\(^{36} \) and neurohumoral tone may modulate repolarization, whereas myocardial stretch may also modulate action potential morphology.\(^{37} \) Larger studies should therefore address whether RPA reflects electrical instability independently of such pathology. Finally, we acknowledge that ours is a small study and that additional work in larger groups of patients is required to validate our results.

### Conclusions

In patients with the substrates for VT, increasingly premature single extrastimuli increase the magnitude of RPA, reverse its phase, and cause it to be distributed later within repolarization. These results suggest that a single premature extrastimulus that fails to induce VT may still produce proarrhythmic effects in susceptible individuals. Measuring such proarrhythmic preconditioning may have implications for dynamically tailoring therapy to arrhythmic susceptibility.

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