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Electrical Restitution and Spatiotemporal Organization During Ventricular Fibrillation

Mark L. Riccio, Marcus L. Koller, Robert F. Gilmour, Jr

Abstract—Despite recent advances in our understanding of the mechanism for ventricular fibrillation (VF), important electrophysiological aspects of the development of VF still are poorly defined. It has been suggested that the onset of VF involves the disintegration of a single spiral wave into many self-perpetuating waves. It has been further suggested that such a process requires that the slope of the electrical restitution relation be ≥ 1 . The same theory anticipates that a single spiral wave will be stable (not disintegrate) if the maximum slope of the restitution relation is < 1 . We have shown previously that the slope of the restitution relation during rapid pacing and during VF is ≥ 1 in canine ventricle. We now show that drugs that reduce the slope of the restitution relation (diacetyl monoxime and verapamil) prevent the induction of VF and convert existing VF into a periodic rhythm. In contrast, a drug that does not reduce the slope of the restitution relation (procainamide) does not prevent the induction of VF, nor does it regularize VF. These results indicate that the kinetics of electrical restitution is a key determinant of VF. Moreover, they suggest novel approaches to preventing the induction or maintenance of VF. (*Circ Res.* 1999;84:955-963.)

Key Words: restitution ■ action potential duration ■ ventricular fibrillation ■ defibrillation

Over the past decade, substantial experimental support has accumulated for the idea that the onset of ventricular fibrillation (VF) is associated with the breakup of a single spiral wave or a pair of counter-rotating spiral waves into multiple wavelets.¹⁻³ However, the process(es) by which spirals may break and cause VF has not been characterized completely. It has been proposed that the breakup of spiral waves is precipitated by oscillations of action potential duration (APD) that are of sufficiently large amplitude to cause conduction block along the spiral wavefront.^{4,5} This conjecture builds on previous work, beginning with the studies of Nolasco and Dahlen,⁶ showing that the slope of the electrical restitution relation determines certain dynamical behavior that may be relevant to the development of VF. In particular, if the restitution relation contains a region of slope ≥ 1 , APD alternans is possible.^{6,7} As we and others have shown previously, induction of APD alternans can be the initial step in a period-doubling sequence that culminates in chaotic dynamics.⁷⁻¹⁰ Such a process could lead to destabilization of wavefronts and the formation of reentrant waves.¹¹

In many experimental studies, the slope of the restitution relation, when determined using standard S1S2 protocols, has been reported to be < 1 .¹² This observation would seem to preclude the breakup of spiral waves secondary to the development of APD alternans. However, we have demonstrated recently that, although the slope of the restitution relation determined using a standard S1S2 protocol is < 1 in canine ventricle, the slope of the restitution relation during

rapid pacing and during VF is ≥ 1 .¹³ If a steep slope of the electrical restitution relation is a prerequisite for VF, then a reduction of the restitution slope should prevent the development of VF. This effect would be manifest both as an inability to induce VF and as a conversion of existing VF into a periodic rhythm.

To test this hypothesis, we did the following: (1) identified drugs that reduced the slope of the restitution relation, (2) tested whether such drugs prevented the induction of VF, (3) tested whether such drugs converted existing VF into a periodic rhythm, and (4) compared the effects of drugs that reduced the slope of the restitution relation with a drug that did not reduce the restitution slope. The results of these studies support the contention that the slope of the restitution relation is an important determinant of VF. Consequently, the kinetics of restitution may be an appropriate target for interventions to prevent VF.

Materials and Methods

All experiments were approved by the Institutional Animal Care and Use Committee of the Center for Research Animal Resources at Cornell University. A total of 29 dogs were used for the study.

Two-Dimensional Preparations: Data Acquisition

Adult mongrel dogs of either sex, weighing 10 to 30 kg, were anesthetized with 390 mg/mL pentobarbital sodium (Fatal-Plus; Vortech Pharmaceuticals; 86 mg/kg IV), and their hearts were excised rapidly and placed in cool Tyrode solution. Thin (≈ 2 -mm-thick) sections of endocardium measuring 10×20 mm were excised from either ventricle and pinned to the bottom of a Plexiglas

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chamber. The preparations were superfused with oxygenated Tyrode solution at a rate of 15 mL/min. The composition of the Tyrode solution (in mmol/L) was: MgCl_2 0.5, NaH_2PO_4 0.9, CaCl_2 2.0, NaCl 137.0, NaHCO_3 24.0, KCl 4.0, and glucose 5.5. The Tyrode solution was bubbled with 95% O_2 and 5% CO_2 . The PO_2 was 400 to 600 mm Hg, the pH was 7.35 ± 0.05 , and the temperature was $37.0 \pm 0.5^\circ\text{C}$.

Initially the fibers were stimulated during a recovery period of at least 60 minutes at a basic cycle length (BCL) of 500 ms. Rectangular pulses of 2 ms duration and 2 to 3 times the diastolic threshold voltage were delivered through polytetrafluoroethylene (Teflon)-coated bipolar silver electrodes using a computer-controlled stimulator. Transmembrane recordings were obtained using standard microelectrode techniques.^{9,10} The recordings were sampled at 5000 Hz with 12-bit resolution using custom-written data acquisition programs. Offline data analysis was performed using programs written in MATLAB 5.2.

Two-Dimensional Preparations: Dynamic and Standard Restitution Protocols

The objective of these experiments was to identify drugs that either did or did not reduce the slope of the restitution relation at the cycle lengths typically encountered during VF. The first drug tested was 2,3-butanedione monoxime (or diacetyl monoxime; DAM), a drug that is used to suppress contraction during optical mapping.^{14,15} Although previous studies have indicated that DAM does not alter the kinetics of restitution,¹⁶ restitution kinetics were not examined at short diastolic intervals (DIs), nor were they examined during rapid pacing. DAM has been reported to have several electrophysiological effects,¹⁷ including inhibition of Ca^{2+} current (I_{Ca}).¹⁸ To determine whether the effects of DAM on restitution were related to blockade of I_{Ca} , we also tested the effects of verapamil. Finally, we determined the effects of the standard Class I antiarrhythmic drug procainamide.

The relationship between APD and DI was determined using standard and dynamic restitution protocols.¹³ For the standard restitution protocol, single test pulses (S2) were delivered after every 20th basic pulse (S1) at a BCL (S1S1) of 300 ms. The S1S2 coupling interval was progressively shortened in steps of 10 to 20 ms starting from 300 ms until the premature pulse was blocked. The S1S2 interval was then increased by 20 ms to restore capture and subsequently was shortened in 1- to 2-ms increments until S2 blocked. The duration of the response to S2 was measured at 95% of repolarization (APD_{95}) and was plotted as a function of the preceding DI. The time course of restitution was fit using a sigmoidal function of the type $\text{APD} = a + b / \{1 + \exp[-(\text{DI} - c)/d]\}$.

For the dynamic restitution protocol, the relationship between APD and DI was determined during pacing at a constant BCL. The BCL was shortened from 400 to 200 ms in steps of 50 ms and from 200 ms to the effective refractory period in steps of 5 to 10 ms. At BCL that produced a 1:1 stimulus:response locking, pacing was stopped after steady state had been reached and APD_{95} of the last paced action potential was measured. During APD alternans, pacing was interrupted twice to directly measure APD_{95} of both the long and the short action potentials. The relationship between APD and DI during constant pacing were determined by plotting APD_{95} as a function of DI and the time course of restitution was fit using a sigmoidal function.

The standard and dynamic restitution relations were determined after 15 to 30 minutes of drug superfusion and after 30 to 60 minutes of washout. The maximum slopes of the standard and dynamic restitution curves before and after drug exposure were compared using an ANOVA, followed by the Scheffé F test, to determine statistical significance. $P < 0.05$ was considered significant. In addition, the range of DI over which the slope of the restitution relation was ≥ 1 , which corresponded to the range of DI over which APD alternans occurred, and the magnitude of the APD alternans was determined during control and during drug exposure and was compared using a paired *t* test. The magnitude of APD alternans was defined as the difference between APD_{95} of consecutive action potentials during 2:2 stimulus:response locking.

Three-Dimensional Preparations: Data Acquisition

Adult dogs were anesthetized as described above, and their hearts were excised rapidly and placed in cool Tyrode solution. The circumflex coronary artery or a branch of the right coronary artery was cannulated using polyethylene tubing. To avoid cutting the coronary vessels and creating vents for the perfusate, Tyrode solution was infused into the coronary artery, and the approximate area of perfusion was identified by blanching of the epicardial surface. A transmural section of tissue 3 to 5 mm larger than the perfused area was then excised. Depending on the size of the heart, the size of the excised segment measured 30 to 50 mm in width, 30 to 90 mm in length, and 10 to 18 mm in depth. The wet weights of the preparations varied from 18.7 to 88.5 g. The preparation was suspended in a Plexiglas chamber with the epicardial surface facing up, where it was both perfused via the coronary artery and superfused with normal Tyrode solution. The flow rates of the perfusate and superfusate were constant at 35 mL/min. Perfusion pressure was 50 to 80 mm Hg, and the temperature was 37.0°C to 38.0°C .

In the initial series of experiments ($n=5$), epicardial activation was monitored using 5 unipolar electrodes made from polytetrafluoroethylene-coated silver wire. In subsequent experiments ($n=24$), epicardial electrical activity was mapped using arrays of 16 or 30 monophasic action potential (MAP)-type recording electrodes, supplemented by 1 to 4 floating glass microelectrodes. The MAP-type electrodes consisted of a silver wire insulated with polytetrafluoroethylene except at the tip that was threaded through a 15-mm-long sheath of 1/8-inch-diameter heat-shrink wrap. A 10-mm-long segment of the sheath was reduced in diameter using moderate heat until it fit snugly around the wire.

The 30-electrode MAP array was mounted on a plastic platform drilled with a 6×5 matrix of holes having 5-mm spacing. The 16-electrode array was arranged linearly with 1.5-mm spacing between the electrodes, using the concept of a contour gauge.¹⁹ The electrodes were held in line by 2 plastic strips screwed together at their ends. As with the 30-electrode array, the tension on the electrodes was such that the electrodes could be moved up and down individually. The MAP arrays were lowered onto the epicardial surface of the preparation using a micromanipulator. The electrodes were then adjusted as necessary until a stable MAP signal was obtained. If an electrode became dislodged during the experiment, it was adjusted to reestablish the MAP signal. The signals from each of the recording sites were referenced to a pellet electrode in the superfusate.

The electrogram, MAP, and transmembrane action potential recordings were displayed on a storage oscilloscope and a thermal array recorder and were sampled at 1250 Hz with 12-bit resolution. The electrogram and MAP signals were high-pass (cutoff=0.15 Hz) and low-pass filtered (cutoff=600 Hz). Records of 4- to 7-second duration were obtained every 20 to 40 seconds during the course of the experiment. Online and offline data analyses were performed using programs written in MATLAB 4.2c.

Three-Dimensional Preparations: Experimental Protocols

Two sets of experiments were performed. In one set, the effects of DAM (20 mmol/L; $n=4$), verapamil (2 $\mu\text{mol/L}$; $n=4$), and procainamide (10 $\mu\text{g/mL}$; $n=4$) on the induction of VF were determined, to test whether drugs that reduced the slope of the dynamic restitution relation prevented the induction of VF, whereas a drug that did not reduce the slope of the dynamic restitution relation did not prevent the induction of VF. In another set of experiments, the effects of DAM (15 mmol/L, $n=5$, or 20 mmol/L, $n=6$), verapamil (2 $\mu\text{mol/L}$; $n=5$), and procainamide (10 $\mu\text{g/mL}$; $n=5$) on spatiotemporal organization during VF were determined, to test whether drugs that reduced the slope of the dynamic restitution relation increased organization during VF, whereas a drug that did not reduce the slope of the dynamic restitution relation had no significant effect on organization during VF.

In the first set of experiments, the hearts were paced initially at a BCL of 800 ms using a bipolar stimulating electrode placed on the

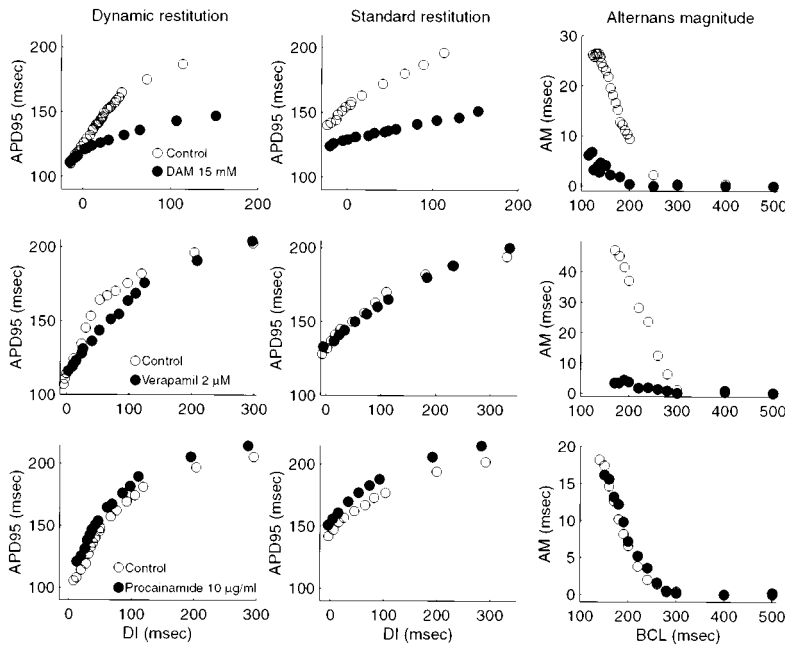


Figure 1. Effects of drugs on dynamic restitution, standard restitution, and magnitude of APD alternans in canine endocardium. Results are shown for DAM (15 mmol/L, top panels), verapamil (2 μ mol/L, middle panels), and procainamide (10 μ g/mL; bottom panels).

epicardial surface. MAP recordings were obtained from the epicardium using the 16-electrode linear array. After a 15-minute equilibration period, the pacing cycle length was shortened progressively, using the same protocol described above for determination of the dynamic restitution relation. During control, shortening the pacing cycle length induced alternans of MAP duration, which culminated in the induction of VF in all preparations ($n=21$). The dynamic restitution relation and incidence of VF induction during control were compared with those obtained after 30 minutes of exposure to DAM, verapamil, or procainamide. After drug exposure, the pacing cycle length was shortened progressively until VF was initiated or until a 2:1 stimulus:response ratio occurred.

For the second set of experiments, the hearts once again were paced initially at a BCL of 800 ms. MAP recordings were obtained using the 16-electrode linear array or the 30-electrode matrix array. After a 15-minute equilibration period, the pacing cycle length was shortened progressively until VF was induced. Ten to 30 minutes after VF had been induced, DAM, verapamil, or procainamide was added to the perfusate and superfusate. The effects of the drugs on spatiotemporal organization were determined during 30 minutes of drug exposure and during 30 to 120 minutes of washout.

Three-Dimensional Preparations: Data Analysis

For analysis of the dynamic restitution relation, MAP duration was measured to 80% of repolarization. Measurements were obtained from all stable MAP recordings, which ranged from 9 to 16 recordings for any given recording. For each pacing cycle length, MAP durations for a given lead were averaged, unless alternans of MAP duration occurred, in which case the longer and shorter MAP durations were averaged separately. From these data, the maximum magnitude of MAP alternans was determined. The magnitudes of maximum MAP duration alternans for each preparation were averaged and were compared using an unpaired *t* test to determine statistically significant differences between control and drug treatment.

To assess the degree of temporal organization during VF and suspected spiral wave reentry, the MAP and action potential data were analyzed using frequency spectral analysis. For each record, the 8 MAP recordings with the largest amplitudes, as assessed at 25 minutes of drug washin, were selected for analysis. Frequency power spectrums for each recording were estimated using the average absolute value (ie, squared-magnitude) of the fast Fourier transforms (FFTs) of 4 Hanning-windowed, 35% overlapped data segments of 1024 samples each. The results subsequently were averaged for all

leads to generate a composite spectrum. To examine temporal changes quantitatively, the average frequency and variance were calculated for the composite spectrum of each record. For these calculations, frequencies <2 Hz and >35 Hz were excluded from the analysis. The variance was calculated as the square root of the SD of the composite spectrum normalized by the maximum power of that spectrum. Variances and mean frequencies for control versus drug treatment were then compared using a paired *t* test.

To provide a qualitative assessment of spatial organization during VF and suspected spiral wave reentry, temporal stacks of data from the 16-MAP electrode linear array were constructed, using a procedure similar to that described by Witkowski et al.³ The MAP recordings were differentiated, with negative values assigned a value of 0, and smoothed using an 8-tap moving average filter. Data for each lead subsequently were normalized according to the maximum value of that lead. The results were imaged over the range (0–0.8) by mapping them to a 255-level grayscale, with the lower and upper bounds being represented by black and white, respectively.

Results

Effects of Drugs on Electrical Restitution in the 2-Dimensional Preparations

During control, the mean maximal slope of the dynamic restitution relation was >1 in all 3 groups of fibers ($n=24$). The steep restitution slope was associated with induction of persistent APD alternans at $BCL < 235 \pm 30$ ms. DAM (15 mmol/L; $n=10$) reduced the maximal slopes of the dynamic and standard restitution relations, as indicated by the example shown in Figure 1 and the summary data in Table 1. The effects of DAM on dynamic restitution were dose dependent over a range of 5 to 20 mmol/L and were reversed completely after 30 minutes of washout (not shown).

Although DAM reduced the maximum slope of the sigmoidal fit to <1 , APD alternans persisted, albeit at a greatly reduced magnitude (Figure 1). Thus, small regions of slope=1 occurred after DAM exposure, despite the fact that the slope of the overall fit was <1 . To better characterize the effects of DAM on dynamic restitution, the range of DI over which alternans occurred and the magnitude of the alternans

TABLE 1. Effects of Drugs on the Dynamic and Standard Restitution Relations*

Drug	Dynamic Slope	AM, ms	DI Range, ms	Standard Slope
DAM				
Control	1.06±0.25	24±10	71±16	0.48±0.20
DAM (n=10)	0.65±0.33†	11±5†	49±22†	0.20±0.13†
Verapamil				
Control	1.02±0.17	24±13	79±23	0.51±0.06
Verapamil (n=7)	0.72±0.23†	4±1†	30±22†	0.41±0.12‡
Procainamide				
Control	1.02±0.17	22±11	75±36	0.54±0.20
Procainamide (n=7)	0.94±0.17	20±11‡	54±22‡	0.38±0.18‡

*Data are given as mean±SD; AM indicates alternans magnitude.

† $P<0.01$; ‡ $P<0.05$ vs control.

also were quantified. DAM reduced the range over which APD alternans occurred from 71 ± 16 to 49 ± 22 ms and reduced the maximum magnitude of APD alternans from 24 ± 10 to 11 ± 5 ms ($P<0.01$) (Table 1).

Verapamil ($2\text{ }\mu\text{mol/L}$; $n=7$) also reduced the maximal slopes of the dynamic and standard restitution relations (Figure 1 and Table 1). In addition, verapamil markedly decreased the maximal amplitude of APD alternans and the range of DI over which alternans occurred (Table 1). In contrast, procainamide ($10\text{ }\mu\text{g/mL}$; $n=7$) did not significantly alter the maximal slope of the dynamic restitution relation (Figure 1 and Table 1). However, procainamide reduced the slope of the standard restitution relation (Table 1). In addition, procainamide slightly, but significantly, reduced the maximal amplitude of APD alternans and the range of DI over which alternans occurred (Table 1). The latter effects resulted from the development of 2:1 conduction block at longer cycle lengths in the presence of procainamide than during control.

Effects of Drugs on the Induction of VF in 3-Dimensional Preparations

Progressive shortening of the pacing cycle length during control induced an alternans of MAP duration, the magnitude of which increased at the shortest pacing cycle lengths to a maximum of 14.0 ± 2.2 ms. After 30 minutes of exposure to

DAM (20 mmol/L ; $n=4$), the magnitude of MAP duration alternans was significantly reduced (to 2.4 ± 0.8 ms; $P<0.05$ versus control). MAP duration alternans also was reduced (to 1.3 ± 0.6 ms; $P<0.05$ versus control) after exposure to verapamil ($2\text{ }\mu\text{mol/L}$; $n=4$). In contrast, the magnitude of MAP alternans was not significantly affected (13.2 ± 3.1 ms; $P=\text{NS}$ versus control) by 30 minutes of exposure to procainamide ($10\text{ }\mu\text{g/mL}$; $n=4$). After exposure to DAM or verapamil, VF was not induced at any pacing cycle length in any of the preparations. In contrast, VF was induced in all 4 preparations after exposure to procainamide.

Effects of Drugs on Spatiotemporal Organization During VF

Figure 2 shows the effects of DAM (15 mmol/L) on microelectrode and unipolar electrogram recordings during VF in a left ventricular preparation. During the initial exposure to DAM, VF progressively regularized into a stable periodic rhythm, whereas after DAM washout, VF recurred. A second exposure to DAM restored the periodic rhythm. In other preparations, VF was stable for at least 60 minutes in the absence of drug exposure.

The progressive increase in temporal organization during DAM exposure also was apparent in the composite frequency spectrum, as shown in Figure 3 for a different experiment. During VF, a wide range of frequencies was present, whereas after DAM exposure, the frequency spectra were dominated by single peak near 14 Hz. In addition, the variance of the spectra was reduced with time of exposure to DAM. The effects of 20 mmol/L DAM on the average frequency and the variance of the frequency spectrum during VF are summarized in Table 2.

Verapamil had a similar effect to DAM on spatiotemporal organization during VF in all 5 preparations studied (Figures 4 through 6). Activation became more synchronous with time of exposure to verapamil, resulting in a periodic activation pattern (Figure 4). In addition, verapamil reduced the variance of the composite frequency spectra (Figure 5 and Table 2). As shown in Figure 6, activation along the 16-electrode linear array was largely asynchronous during VF, although some instances of synchronous or consecutive activation did occur. With increasing time of exposure to verapamil, activation became more organized, culminating in a periodic

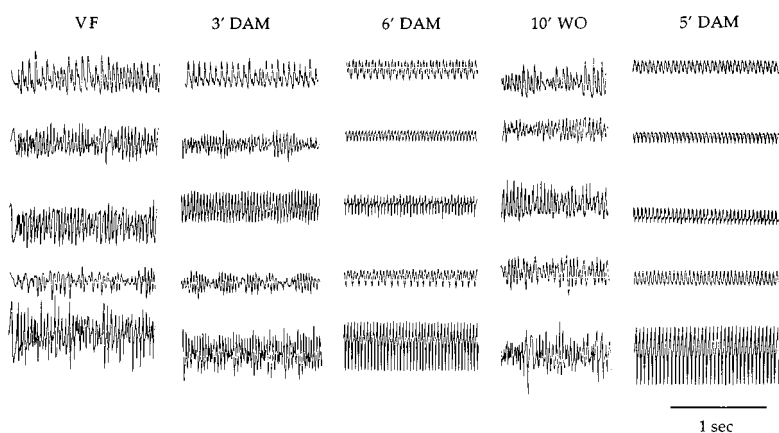


Figure 2. Effects of DAM (15 mmol/L) on VF in arterially perfused canine left ventricle. Action potential (top trace) and unipolar electrogram recordings (4 lower traces) are shown after 20 minutes of VF had elapsed in the absence of drug exposure (VF), after 3 and 6 minutes of exposure to DAM (3' DAM and 6' DAM, respectively), after 10 minutes of DAM washout (10' WO), and after 5 minutes of a second exposure to DAM (5' DAM). The same microelectrode impalement was not maintained throughout, and impalement quality varied. Vertical bar indicates 100 mV for action potential recordings and 10 mV for electrogram recordings.

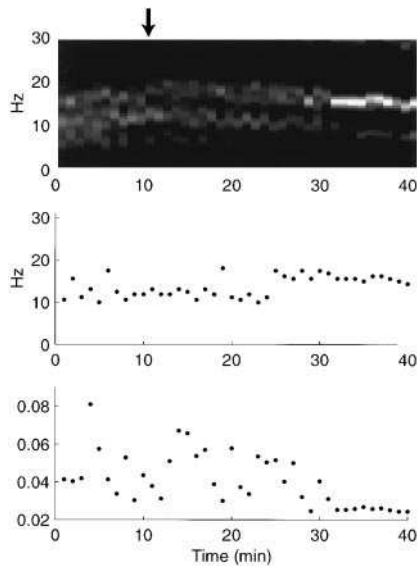


Figure 3. Effects of DAM on average FFT spectra (top panel), mean activation frequency (middle panel), and variance of the FFT spectra (bottom panel) during VF in arterially perfused canine left ventricle. Variance of the spectra is represented by the moving average of 4 spectra. DAM exposure began after 10 minutes of VF had elapsed (arrow, top) and continued for another 30 minutes. Time 0 indicates the initiation of VF.

rhythm with a fixed frequency and activation sequence. In 4 of the 5 preparations, VF was restored after 60 to 120 minutes of verapamil washout.

In contrast to the effects of DAM and verapamil, procainamide did not significantly increase spatiotemporal organization during VF (Figures 7 and 8). Procainamide shifted the frequency distribution during VF to lower frequencies and reduced the mean frequency (Figure 8 and Table 2), consistent with the increase in the mean VF cycle length reported by Kwan et al.²⁰ However, procainamide did not regularize VF, as illustrated by the lack of synchronous activation in the MAP recordings (Figure 7) and the lack of an effect on the variance of the FFT spectra (Figure 8 and Table 2).

Discussion

New Findings

In this study, verapamil and DAM, 2 drugs that reduced the slope of the dynamic restitution relation, prevented the

TABLE 2. Effects of Drugs on VF Frequency Spectrum*

Drug	Variance	Mean Frequency, Hz
DAM		
Control	0.0622±0.0070	11.23±3.81
DAM (n=6)	0.0280±0.0014†	12.87±4.10
Verapamil		
Control	0.0634±0.0138	10.34±4.19
Verapamil (n=5)	0.0344±0.0032†	10.46±3.97
Procainamide		
Control	0.0546±0.0105	11.78±2.33
Procainamide (n=5)	0.0492±0.0084	11.06±2.30‡

*Data are given as mean±SD.

† $P<0.01$; ‡ $P<0.05$ vs control.

induction of VF by rapid pacing and converted existing VF into a periodic rhythm. In contrast, procainamide, a drug that did not reduce the slope of the dynamic restitution relation, failed to prevent the induction of VF by rapid pacing. In addition, procainamide produced only minor changes in spatiotemporal organization during existing VF. These results support the hypothesis that a steeply sloped restitution relation is a prerequisite for the development and maintenance of VF.

Role of Electrical Restitution in VF

Our study was motivated by the suggestion that a steep slope of electrical restitution predisposes to the breakup of single spiral waves into multiple spiral waves,^{4,5} a process that may account for the transition from ventricular tachycardia to VF.¹⁻³ We found this hypothesis attractive, despite the fact that it has been discounted by several investigators on the grounds that the slope of the restitution relation, when determined using standard S1S2 protocols, typically is <1 .¹² In addition, the theory predicts that a single spiral wave will disintegrate into multiple spiral waves in 2-dimensional myocardium, yet experimental observations have indicated that spiral waves in ostensibly normal 2-dimensional myocardium are remarkably stable.^{14,15}

These observations have spawned alternative explanations for the development of VF in 3-dimensional myocardium. For example, it has been proposed that a transmural gradient of excitability²¹ or rotational anisotropy^{22,23} destabilizes the filament of a 3-dimensional spiral wave (vortex), leading to the creation of multiple vortices. On the other hand, Panfilov⁵ has suggested that spiral wave breakup in 3-dimensional myocardium, as in 2-dimensional myocardium, requires a steep slope of restitution, although the restitution slope need not be as steep in 3 dimensions as in 2 dimensions. The results of the present study lend further support to the idea that a steeply sloped restitution relation is required for the development of VF in 3-dimensional myocardium.

Although our studies were designed to determine whether APD restitution is an important determinant of VF, they were not designed to determine whether it is the sole determinant. Other electrophysiological properties, such as conduction velocity (represented by the diffusion relation in computer models) and cell coupling (represented by a coupling coefficient), may contribute significantly to the development of VF.^{4,5} In addition, transmural fiber rotation^{22,23} and the variation of cellular electrical properties in different layers of myocardium^{21,24} probably play important roles in determining the activation sequences in the intact heart. Wall thickness, heart size, and the distribution of specialized conducting tissue also are potential modulators of VF (see Reference 25).

Of these potential determinants for the behavior of VF, those most likely to be affected by drugs are conduction velocity and cell coupling. The contributions of changes in conduction velocity or cell coupling to the effects of the drugs we have tested thus far presently are unknown. DAM has been reported to decrease upstroke velocity¹⁶ and, on that basis, might reduce conduction velocity. However, procainamide also decreases upstroke velocity,²⁶ but does not suppress VF, whereas verapamil has little effect on upstroke

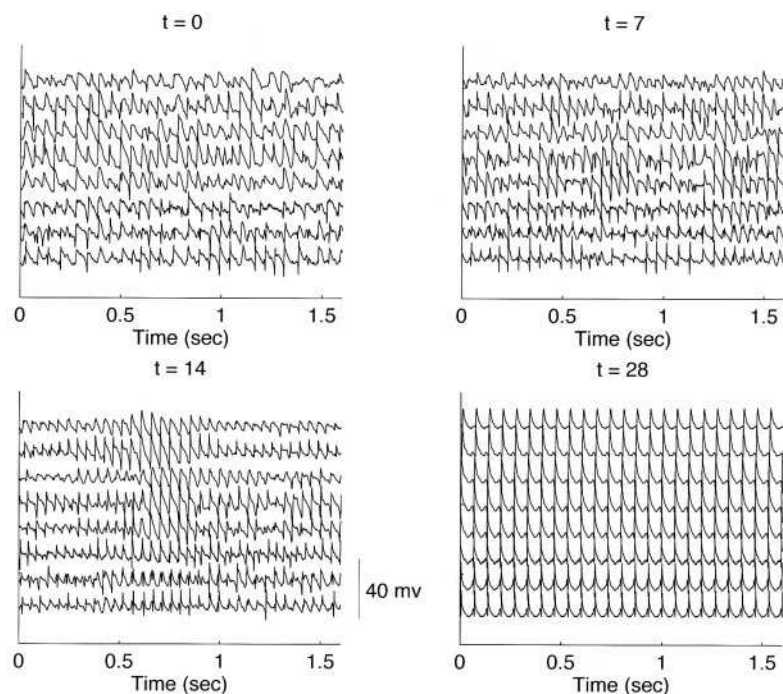


Figure 4. Effects of verapamil ($2 \mu\text{mol/L}$) on VF in arterially perfused canine left ventricle. Examples of 8 MAP recordings are shown after 30 minutes of VF had elapsed in the absence of drug exposure ($t=0$) and after 7, 14, and 28 minutes of verapamil exposure.

velocity,²⁷ yet it suppresses VF. Alternatively, verapamil and DAM may suppress VF via alterations of $[\text{Ca}^{2+}]_i$, as suggested by previous studies in which calcium channel blockers and low $[\text{Ca}^{2+}]_o$ converted VF to ventricular tachycardia^{28–30} (although not all studies have found such an effect^{31,32}). Suppression of oscillations in $[\text{Ca}^{2+}]_i$, secondary to blockade of I_{Ca} , would be expected to reduce APD alternans,³³ yet in the studies of Merrillat et al,³⁰ verapamil suppressed VF but

ryanodine did not. Further studies are needed to clarify this issue.

Significance

Historically, therapy for the prevention of sudden cardiac death has been predicated on the idea that frequent ventricular ectopy, in particular ventricular tachycardia, is a harbinger of VF.³⁴ Accordingly, drugs that suppress inducible or spontaneously occurring ventricular tachycardia are expected to prevent sudden death. However, a paradox has arisen in which a class of drugs that is effective for the suppression of ventricular tachycardia, the Class I antiarrhythmic drugs, does not prevent sudden death.³⁵ In contrast, other classes of drugs that are not particularly effective for the suppression of most forms of ventricular tachycardia reduce mortality from sudden death. These drugs include β -adrenergic receptor antagonists³⁶ and, to a lesser extent, calcium channel antagonists.³⁷

Our observation that the slope of the restitution relation is an important determinant of VF could have significant implications for the pharmacological therapy of sudden death. Drugs that reduce the slope of the restitution relation would be expected to prevent the development of VF but would not be expected to suppress ventricular tachycardia, if ventricular tachycardia is caused by some variant of spiral wave reentry.^{14,15} In fact, such drugs might stabilize ventricular tachycardia. Conversely, drugs that do not reduce the slope of the restitution relation would not be expected to prevent VF, although they might suppress ventricular tachycardia, perhaps via a mechanism that does not involve alteration of restitution kinetics (eg, slowing of conduction or prolongation of refractoriness).

In our studies, reduction of the restitution slope was accomplished using drugs that also significantly reduced force development. If the dose-response relationships for the

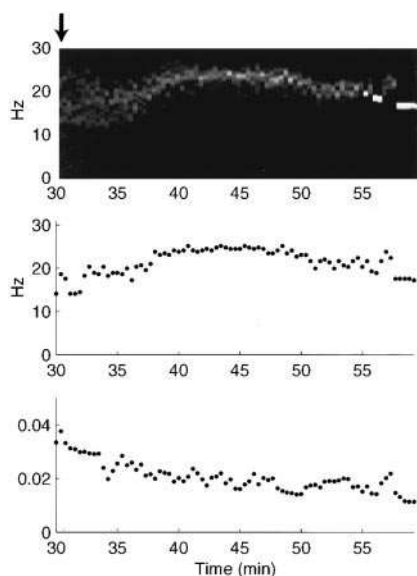


Figure 5. Effects of verapamil on average FFT spectra (top panel), mean activation frequency (middle panel) and variance of the FFT spectra (bottom panel) during VF in arterially perfused canine left ventricle; same preparation as described in Figure 4. Verapamil exposure began after 30 minutes of VF had elapsed (arrow, top) and continued for another 30 minutes, at which time washout was started (not shown). Data are shown beginning at 30 minutes of VF.

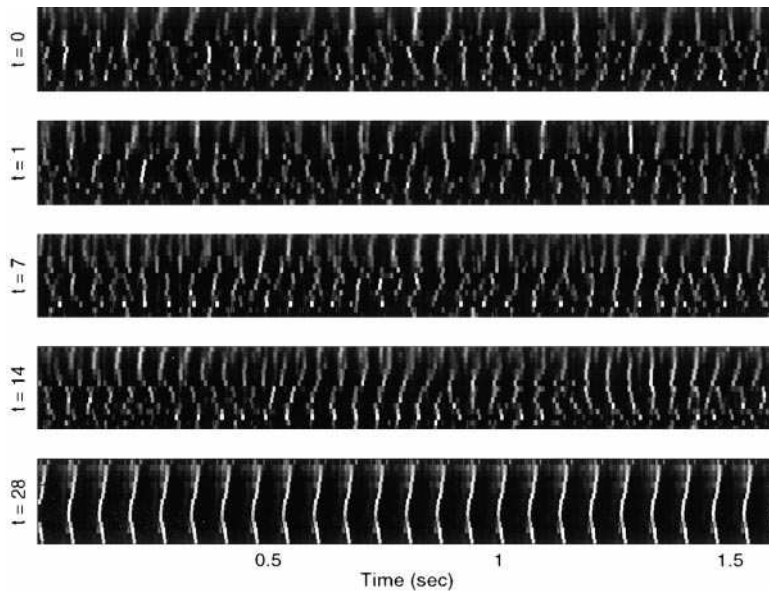


Figure 6. Temporal stack of MAP data during VF in arterially perfused canine left ventricle; same preparation as in Figures 4 and 5. Activation along the 16-MAP electrode linear array is illustrated as a function of time. The upper 2 recordings ($t=0$ and $t=1$) were obtained consecutively during VF in the absence of drug exposure. The lower recordings were obtained after 7, 14, and 28 minutes of exposure to verapamil.

effects of these drugs on VF and on inotropy are similar, then blockade of I_{Ca} would not be a clinically useful method of preventing VF. Consequently, other strategies for reducing the slope of the restitution relation may need to be developed.

Limitations

Although the results of the present study are consistent with the hypothesis that a steep slope of electrical restitution predisposes to the breakup of a single spiral wave into multiple spiral waves, proof of that hypothesis would require a demonstration of spiral wave formation and disintegration in the intact heart. The latter would, in turn, require detailed 3-dimensional maps of electrical activation and repolarization, which are not at present techni-

cally feasible. In the absence of such maps, it remains possible that the regularization of activation during VF observed in our study resulted from a phenomenon other than the coalescence of many spiral waves into 1. For example, if VF is caused by a single spiral wave that creates an irregular activation pattern because of meander³⁸ or block of fibrillatory impulses into certain regions of the heart,³⁹ then regularization of VF could reflect the anchoring of the spiral wave or, alternatively, the abolition of conduction block, perhaps secondary to a reduction in heterogeneity of refractoriness.

The results of our study also may have been influenced by the use of a perfused segment of ventricle, which necessarily was bordered by a region of potentially ische-

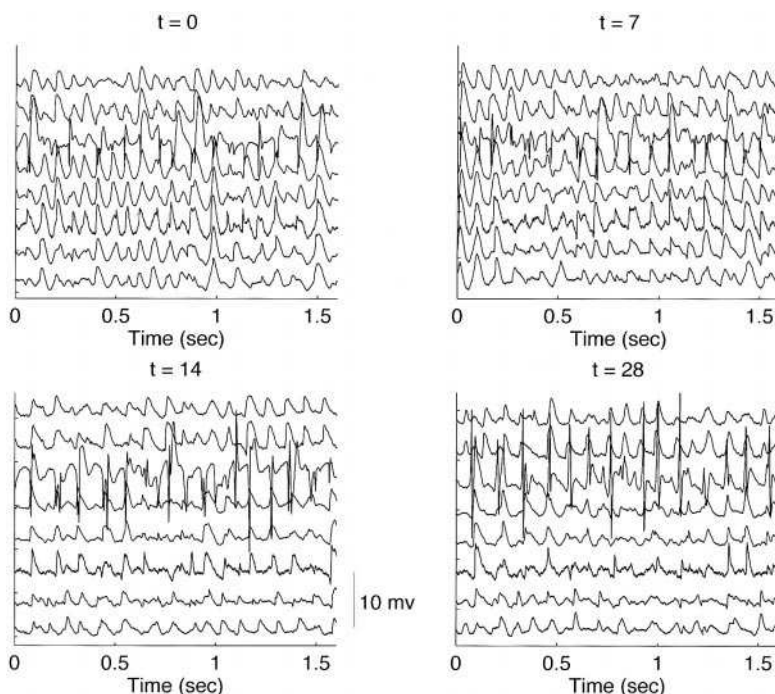


Figure 7. Effects of procainamide ($10 \mu\text{g/mL}$) on VF in arterially perfused canine left ventricle. Examples of 8 MAP recordings are shown after 45 minutes of VF had elapsed in the absence of drug exposure ($t=0$) and after 7, 14, and 28 minutes of procainamide exposure.

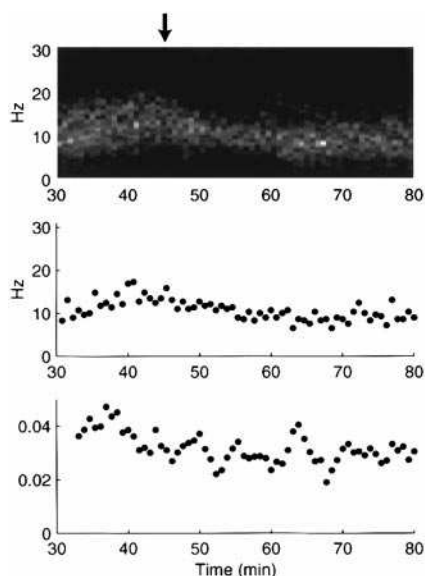


Figure 8. Effects of procainamide on average FFT spectra (top panel), mean activation frequency (middle panel), and variance of the FFT spectra (bottom panel) during VF in arterially perfused canine left ventricle; same preparation as in Figure 7. DAM exposure began after 45 minutes of VF had elapsed (arrow, top) and continued for another 30 minutes, at which time washout was started (not shown). Data are shown beginning at 30 minutes of VF.

mic tissue. Given that the flow rate of coronary perfusate was somewhat lower than that present in vivo, there is a possibility that the bulk of the preparation also was ischemic. However, the lack of a significant contribution of ischemia to the results was suggested by the observation that alternans of APD was present before the induction of VF. Recently, we have shown that moderate hyperkalemia ($[KCl]=6$ to 8 mmol/L) flattens the restitution relation and reduces the magnitude of APD alternans.⁴⁰ Accordingly, if ischemia were present in our preparations, we would expect a similar suppression of APD alternans, which we did not observe.

Finally, the results of studies in canine heart may not be directly applicable to other species, in which differences in heart size and in restitution properties¹² may affect the contribution of restitution to the development of VF. Nevertheless, the demonstration that verapamil increases spatiotemporal organization during VF in the rabbit heart^{28,29} supports the idea that the slope of the restitution relation is an important determinant for the development of VF across species.

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