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Optical Mapping of Drug-Induced Polymorphic Arrhythmias and Torsade de Pointes in the Isolated Rabbit Heart

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Objectives. **This study sought to 1) test the hypothesis that in the setting of bradycardia and drug-induced action potential prolongation, multiple foci of early afterdepolarizations (EADs) result in beat to beat changes in the origin and direction of the excitation wave front and are responsible for polymorphic arrhythmias; and 2) determine whether EADs may initiate nonstationary reentry, giving rise to the typical torsade de pointes (TDP) pattern.**

Background. **In the past, it has been difficult to associate EADs or reentry with the undulating electrocardiographic (ECG) patterns of TDP.**

Methods. **A voltage-sensitive dye was used for high resolution video imaging of electrical waves on the epicardial and endocardial surface of the Langendorff-perfused rabbit heart. ECG and monophasic action potentials from the right septal region were also recorded. Bradycardia was induced by ablation of the atrioventricular node.**

Results. **Perfusion of low potassium chloride Tyrode solution plus quinidine led to prolongation of the action potential and the QT interval. Eventually, EADs and triggered activity ensued, giving rise to intermittent episodes of polymorphic arrhythmia. In one experiment, triggered activity was followed by a long episode of vortex-like reentry with an ECG pattern characteristic of TDP. However, in most experiments, focal activity of varying origins and propagation patterns was observed. Triggered responses also showed varying degrees of local block. Similar results were obtained with E-4031. Burst pacing both at control conditions and in the presence of quinidine consistently led to vortex-like reentry whose ECG pattern resembled TDP. However, the cycle length of the arrhythmia with quinidine was longer than that for control ([mean ± SEM] 194 ± 12 vs. 132 ± 8 ms, $p < 0.03$).**

Conclusions. **Drug-induced polymorphic ventricular arrhythmias may result from beat to beat changes in wave propagation patterns initiated by EADs or EAD-induced nonstationary reentrant activity. In contrast, burst pacing–induced polymorphic tachycardia in the presence or absence of drugs is the result of nonstationary reentrant activity.**

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Polymorphic ventricular tachycardia is a particularly life-threatening arrhythmia that often precipitates ventricular fibrillation (1). This arrhythmia is characterized by a complex electrocardiographic (ECG) pattern with an irregular QRS configuration and relatively high ventricular rates. Torsade de pointes (TDP) is a polymorphic tachycardia characterized by a continuous twisting of the QRS axis around an imaginary baseline on the ECG (2). TDP occurs more commonly in the presence of QT interval prolongation and is often the result of the administration of certain antiarrhythmic drugs (3). There is wide agreement among investigators that the arrhythmia that develops under such conditions is somehow related to triggered activity (4–6) brought about by early afterdepolarizations (EADs). However, although EADs provide a potential explanation for the initiation of TDP, and possibly other forms of polymorphic tachycardia, it is difficult to relate such a mechanism to the undulating ECG patterns observed during the TDP episodes. Thus, the mechanisms of antiarrhythmic drug-induced polymorphic tachycardias, particularly TDP, remain uncertain. We recently demonstrated that polymorphic tachycardia (7) and ventricular fibrillation (8) induced by burst pacing in the structurally normal rabbit heart are the result of one, two or several coexisting electrical vortices (spiral waves) that drift rapidly throughout the ventricles and give rise to complex patterns of cardiac muscle excitation. Thus, it is tempting to speculate that drifting spiral wave activity may underlie at least some cases of drug-induced polymorphic arrhythmias, including TDP.

The present study sought to clarify the mechanism of drug-induced polymorphic ventricular arrhythmias in the setting of QT prolongation. To that end, we investigated the activation patterns and ECG characteristics of ventricular arrhythmias occurring after periods of bradycardia, as well as during burst pacing. Our results show that drug-induced EADs

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Abbreviations and Acronyms

AVN = atrioventricular node
EAD = early afterdepolarization
ECG = electrocardiogram, electrocardiographic
LAD = left anterior descending coronary artery
LV = left ventricle, left ventricular
MAP = monophasic action potential
RV = right ventricle, right ventricular
TDP = torsade de pointes

occurring at multiple subendocardial sites lead to beat to beat changes in the ventricular activation sequence and results in polymorphic ECG complexes. In addition, if conditions are appropriate, the interaction of two or more wave fronts initiated by EADs at varying sites may initiate drifting spiral wave activity and give rise to typical TDP episodes.

Methods

Langendorff-perfused rabbit heart preparation. All experiments were conducted in isolated Langendorff-perfused rabbit hearts and were in compliance with animal welfare regulations at the State University of New York Health Science Center, Syracuse, New York. New Zealand White rabbits (~2 kg) were anesthetized with sodium pentobarbital (35 mg/kg body weight). The heart was rapidly removed through a thoracotomy and connected to the Langendorff apparatus, and the coronary arteries were continuously perfused through a cannula in the aortic root with warm (37 to 39°C) Tyrode solution saturated with 95% oxygen and 5% carbon dioxide, and buffered to a pH of 7.4, under a pressure head of 70 mm Hg. The following solution was used (mmol/liter): NaCl 130, KCl 4, $NaHCO_3$ 24, NaH_2PO_4 1.2, $CaCl_2$ 1.8, $MgCl_2$ 1.0 and glucose 5.6. Next, the heart was immersed in a rectangular temperature-controlled beaker full of Tyrode solution, which was used as a volume conductor for recording the ECG. Two unipolar ECG electrodes were utilized, and the tip of each electrode was positioned ~5 mm from the lateral side of the heart. Tyrode solution containing the potentiometric dye di-4-ANEPPS (15 μg/ml) was perfused through the coronary arteries for 1 to 2 min. In addition, monophasic action potentials (MAPs) were recorded using a bipolar suction electrode catheter containing a long Ag/AgCl distal electrode and connected to a high input impedance amplifier. The MAP recording electrode was positioned on the right ventricular septal endocardium (frequency range 0.04 to 500 Hz). Data acceptable for MAP analysis were derived from stable recordings only that were judged according to previously established criteria (6). Action potential duration was obtained by measuring the time to 90% repolarization.

High resolution optical mapping system. Details of the optical mapping system have been published elsewhere (7,9,10). Briefly, collimated light from a tungsten–halogen lamp passed through a heat filter and an interference filter (490 nm); excitation light then shined on the epicardial surface of the vertically hanging heart. For epicardial mapping, the anterior epicardial ventricular wall with the left anterior descending coronary artery (LAD) faced the light source and the video camera. In endocardial mapping experiments, the right ventricle (RV) was cut open along the posterior interventricular margin down to the apex. The flap consisting of the anterior and inferior walls of the RV was laid open and its endocardial surface as well as that of the interventricular septum were exposed to the filtered light. A 50-mm objective lens was used to collect the emitted light with a depth of field of ~12 mm. The emitted light was transmitted through an emission filter (645 nm) and to a charge coupled device (CCD) video camera (Cohu 6500). Video images (typically 200 × 200 pixels) were acquired by a frame grabber (Epix) in noninterlace mode with a speed of 120 frames/s (8.33-ms sampling rate). The spatial resolution was ~0.15 mm (7). The frame-grabber board was mounted in a pentium computer (Gateway 386/33), which was also used to process the imaged data. To reveal the signal, the background fluorescence was subtracted from each frame. Low pass spatial filtering (10) was usually applied to improve the signals. All optical recordings were ~6 s in duration. No electromechanical uncoupling agents were used in these experiments.

Definitions. The term *triggered activity* is used herein as a synonym of EADs. EADs are recorded endocardially as single or repetitive MAP discharges occurring during the repolarization phase and are triggered by a single spontaneous or externally induced ventricular depolarization. Prolongation of the action potential duration could be a basis for occurrence of EADs, and EADs occur during either phase 2 or phase 3 of the action potential. The time course of repolarization could be altered in the form of a prolonged plateau before the upstroke of discharge. *Polymorphic arrhythmias* are nonsustained or sustained ventricular arrhythmias whose ECG appearance changes on a beat to beat basis. *TDP* is a rapid paroxystic polymorphic arrhythmia that is characterized by gradual (undulatory) changes in the QRS axis; occasionally, TDP deteriorates into ventricular fibrillation.

Experimental protocols. After 20 min of equilibration, bradycardia was induced by ablation of the atrioventricular node (AVN) using the tip of a portable high temperature cautery (C-Line Accu-Temp, Bristol Myers Squibb). Unless otherwise stated, stimuli were delivered through a coaxial Ag/AgCl bipolar electrode (0.1-mm thick; insulated except for the tip) applied to the endocardial surface of the RV at twice the diastolic threshold. After AVN ablation, the heart usually developed a slow idioventricular rhythm. Alternatively, the heart was paced at basic cycle lengths of 2 to 3 s. We carried out simultaneous optical mapping and ECG and MAP recordings of arrhythmias in two different types of experiment. In the first type, ventricular arrhythmias triggered by idioventricular pacemaker discharges were recorded and analyzed. Alternatively, triggered arrhythmias were induced by a single biphasic pulse. These procedures were repeated for 12 experiments in which either 5 μmol/liter quinidine (n = 6) or 0.5 μmol/liter

E-4031 (n = 6) was perfused. In the second type, we applied burst pacing (cycle length 100 to 200 ms) to the endocardial surface of the RV in the absence and presence of quinidine (n = 8). In some experiments, rapid stimuli were also applied to the left ventricular (LV) endocardium. The number of pulses in the burst was progressively increased until a sustained ventricular tachyarrhythmia was induced.

Isochrone maps. Isochrone maps were generated from filtered video imaging data by analyzing the value of each pixel over time. A point in the time plot was labeled as a wave front if it was the fastest part of the upstroke (i.e., the maximal first derivative). Thresholds helped to eliminate most maxima due to noise. Each isochrone band (either colored or of a given gray level) corresponds to the propagation of the wave front in one 8.3-ms frame (7). To eliminate motion artifacts associated with cardiac contraction, we used the following approaches: 1) sequential subtraction of each frame from the previous one was substituted for the usual background subtraction (7); 2) in selected cases, a motion correction algorithm was used. The motion correction algorithm converted a movie of the contracting heart into a movie of a motionless heart with propagation patterns still intact. A reference image was selected, usually the first frame of the movie. The subsequent movie frames were altered to fit the reference image.

Preparation of drugs. Quinidine sulfate (Sigma) and E-4031 (Eisai) were dissolved in distilled water, each at a concentration of 1 mmol/liter. Freshly prepared stock solutions were added to low KCl (3 mmol/liter) Tyrode solution to make up the final concentrations. Di-4-ANEPPS was dissolved in dimethylsulfoxide (5 mg/ml) and diluted in Ringer solution to make up the final concentration.

Statistical analyses. Results are expressed as mean value ± SEM. Drug effects, frequency dependence and arrhythmia cycle length were compared both within and across hearts using analysis of variance and Fisher protected least significant difference testing. A p value <0.05 was considered statistically significant. The analyses were performed using StatView, version 4.11, for personal computers.

Results

Effects of quinidine and E-4031 on QT interval and MAP duration. To validate our procedures, we determined the effects of quinidine and E-4031 on action potential duration in a group of 12 experiments. Data were obtained after AVN ablation during RV pacing at the basic cycle length of 1,000 ms. Perfusion of quinidine resulted in a significant prolongation of the MAP duration (from 228 ± 18 to 325 ± 17 ms, $p < 0.01$) and QT interval (from 293 ± 19 to 410 ± 54 ms, $p < 0.003$) compared with control conditions (KCl concentration = 3 mmol/liter). Similarly, when E-4031 was added to low KCl (3 mmol/liter) Tyrode solution, the MAP duration increased from 212 ± 8 to 317 ± 25 ms ($p < 0.003$) and the QT interval from 270 ± 8 to 408 ± 26 ms ($p < 0.0001$). We evaluated also the rate dependence of quinidine-induced prolongation of the MAP, at cycle lengths between 200 and 2,000 ms, during control conditions (KCl concentration 3 mmol/liter) and 15 min after the addition of 5 μmol/liter quinidine (data not shown). As expected from previous studies (11), quinidine perfusion led to prolongation of the MAP at all basic cycle lengths, except for 200 ms. The effect was more pronounced at slow rates (i.e., reverse use dependence). A similar use dependence was demonstrated when 0.5 μmol/liter E-4031 was used.

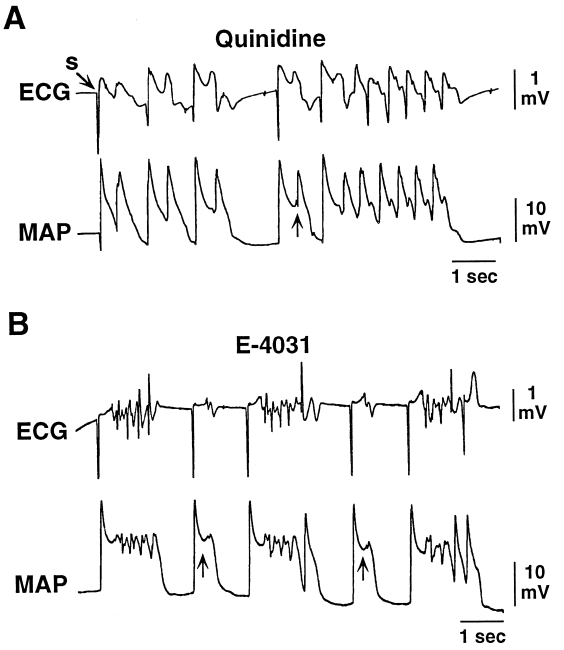

Figure 1. ECG and MAP recordings of polymorphic arrhythmia induced during infusion of 5 μmol/liter quinidine (**A**) and 0.5 μmol/liter E-4031 (**B**) after AVN ablation. In both cases, an escape beat or a beat initiated by a single stimulus to the RV is followed by EADs and triggered activity, giving rise to intermittent episodes of bigeminy or polymorphic activity, or both. **Arrows** indicate slow rising upstrokes of triggered discharges. s = time of application of stimulus.

EADs and polymorphic arrhythmias. *MAP and ECG.* Drug-induced polymorphic arrhythmias were triggered by spontaneously occurring idioventricular escapes or by single ventricular stimuli applied after long (>2 s) periods of quiescence. The mean escape interval after AVN ablation was 1,406 ± 898 ms in 7 of 12 preparations. The remaining five hearts did not demonstrate idioventricular rhythms but showed only sporadic escape complexes at extremely low frequencies. In Figure 1, we illustrate examples of ECG and MAP recordings during perfusion with 5 μmol/liter quinidine (panel A) and 0.5 μmol/liter E-4031 (panel B). Escape beats were sometimes followed by EADs that seemed to occur within or near the site of MAP recording. This occurrence is suggested by the relatively smooth transitions from the diastolic potentials to the relatively slow-rising upstrokes of some of the individual triggered discharges (arrows). However, most frequently the triggering event gave rise to bursts of propagated activity that appeared to be generated more distally, as judged by the sharp transitions between the maximal diastolic poten-

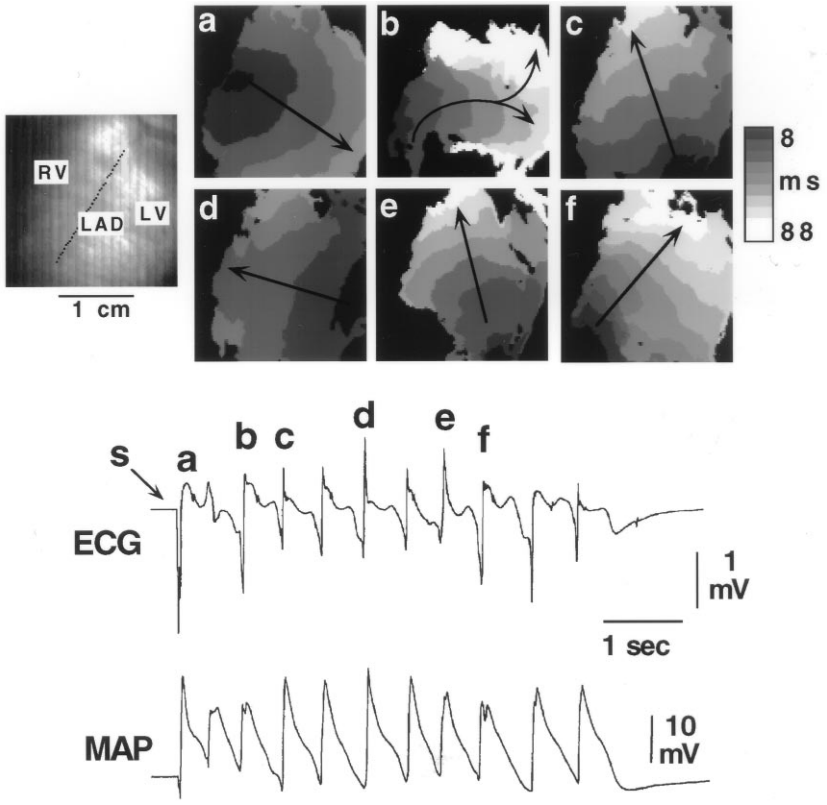

Figure 2. Quinidine-induced polymorphic tachycardia. **Top panel,** Eight-millisecond epicardial isochronal maps of the anterior ventricular surface during an episode of polymorphic arrhythmia induced by perfusion of quinidine. The **leftmost frame** shows the real image of the preparation. Each map corresponds to the ECG deflection and MAP indicated by the respective letter on the ECG (**middle and bottom panels**). **Arrows** indicate the direction of propagation. s = stimulus.

tials and the upstrokes of the individual action potentials in the burst. On the ECG these events were manifest as intermittent episodes of bigeminy or repetitive polymorphic QRS complexes. The mean cycle length of the polymorphic arrhythmia in the quinidine (n = 6) and E-4031 experiments (n = 6) was 424 ± 19 ms (16 episodes) and 372 ± 20 ms (13 episodes), respectively. The duration of the episodes varied from 2.0 to 15 s.

Epicardial mapping. The top panel of Figure 2 shows on the left a snapshot of the fluorescent image of the preparation as seen by the video camera before background subtraction. The image shows the anterior epicardial ventricular wall with the RV on the left, the LV on the right and the LAD in the center, highlighted by a diagonal dotted line. On the right, frames a to f represent consecutive isochronal maps of wave front propagation through the same field obtained during an episode of quinidine-induced polymorphic arrhythmia. Each map corresponds to the interval that followed the QRS complexes, indicated by the respective letter on the ECG (middle panel). For reference, the corresponding RV endocardial MAP tracing is shown in the bottom panel. After a prolonged period of quiescence, a single stimulus (s) was delivered to the endocardial surface of the RV. The ensuing discharge triggered a burst of spontaneous action potentials and resulted in an episode of nonsustained polymorphic arrhythmia; note that in this case, all action potentials seem to have originated outside the MAP recording area. As shown by map a, the first discharge manifested as a breakthrough site (black area) near the center of the free wall of the RV. The wave front thus initiated then propagated radially throughout the epicardial surface and activated the entire anterior wall within <40 ms. Yet, as illustrated by isochronal maps b to f, each of the 10 triggered responses that followed originated at a different site; therefore, the activation pattern changed on a beat to beat basis. Because of the prematurity of the triggered discharges, each wave front generated encountered the ventricular wall at different stages of recovery. Consequently, there were local variations in the ability of each impulse to propagate, as demonstrated by the varying degrees of convergence of the isochrone bands, as well as the presence of relatively large areas of block in some of the maps (black areas at the bottom of maps b and e).

A striking example of nonsustained polymorphic arrhythmia triggered by a single spontaneous escape beat is presented in Figure 3. The data were taken from an experiment in which the heart was continuously perfused with 3 mmol/liter KCl Tyrode solution containing 0.5 μmol/liter E-4031. As shown by the MAP recording from the RV endocardium, the action potential duration of the escape beat was exceedingly large (>1,200 ms), and its plateau was perturbed by relatively small oscillations, each of which corresponded to a QRS complex on the ECG. The oscillations in the MAP grew in amplitude until almost full repolarization ensued. However, repolarization was again interrupted by a closely coupled action potential. The isochronal maps in the top panel reveal the mechanism of the oscillatory activity in the MAP and the resulting bizarre

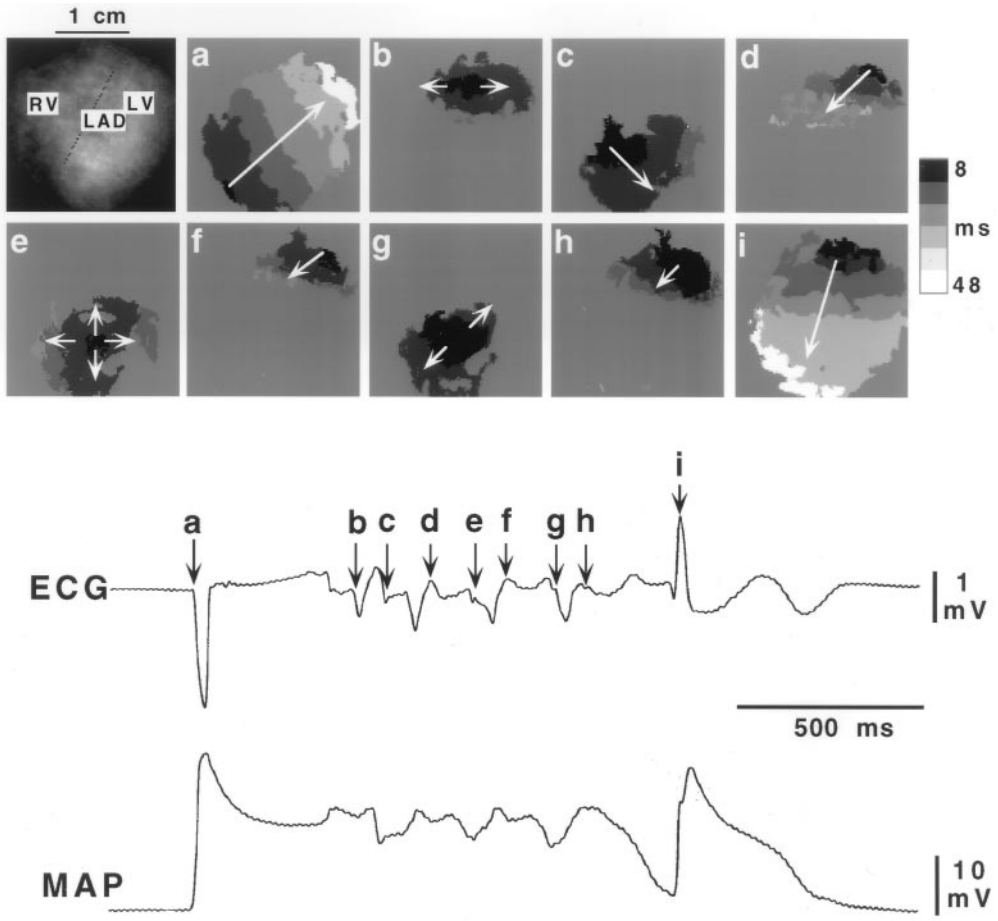

Figure 3. E-4031-induced polymorphic tachycardia. **Top panel,** Epicardial maps obtained from the surface of the anterior LV wall and the RV free wall during an episode of polymorphic arrhythmia induced by coronary perfusion of 0.5 μmol/liter E-4031. **Middle and bottom panels,** ECG and MAP recorded simultaneously during polymorphic tachycardia. The first ECG activity (a) is a ventricular escape beat; b to h represent polytopic triggered activity; beat i apparently occurs at the end of the episode. **Arrows** indicate the direction of propagation.

polymorphic arrhythmia on the ECG. As suggested by the initial breakthrough site (black) in map a, the escape beat probably originated subepicardially near the RV apex. The wave front propagated upward and to the right to activate the whole epicardial surface of both ventricles. Subsequently, after a long pause, a new breakthrough appeared at the base of the LV but was unable to propagate very far in any direction (map b). Immediately thereafter (map c), another breakthrough appeared near the center of the septum; the wave front now moved downward toward the apex of the LV but blocked everywhere else. In the next beat (map d), the site of breakthrough moved to the base of the LV, and activation proceeded downward and to the left but was unable to continue, probably because of refractoriness in the remainder of the ventricle. As shown by the sequential maps in frames e to h, beat to beat alternation in the site of breakthrough continued and in each of the premature beats encountered appreciable block over most of the epicardial surface. Because beat i occurred after an appreciable recovery interval, the wave front now was able to propagate downward from the base of the LV and activate most of the epicardial surface (map i). In summary, the results strongly suggest that in this case, the highly polymorphic pattern observed on the ECG was the result of triggered activity with multiple sites of epicardial breakthrough resulting from EAD-induced early activation and a high degree of functionally determined block.

Endocardial mapping. From the data presented thus far it is difficult to tell whether the beat to beat changes in epicardial propagation are the result of multiple sites of initiation in the subendocardium or whether all action potentials are initiated at a single site, with beat to beat spatial changes in refractoriness leading to multiple sites of epicardial breakthrough. We therefore carried out endocardial mapping of the RV during perfusion with quinidine in an effort to track the origin of each individual beat to specific foci. Results of one experiment during perfusion with quinidine are presented in Figure 4. In the top panel, the leftmost frame shows a picture of the preparation. The RV septum is at the center, and the RV free wall is at the right. The MAP electrode was located on the left (asterisk) near the insertion of the anterior papillary muscle. The ECG (middle panel), obtained by placing each lead electrode at either side of the plane, shows an intermittent arrhythmia with salvos of five to six QRS complexes, each corresponding to an action potential in the monophasic recording (bottom panel). In this case, the arrhythmic episode showed an undulating pattern that resembled TDP. Unfortunately, the relatively poor quality of the MAP trace prevented us from establishing whether any of the spontaneous dis-

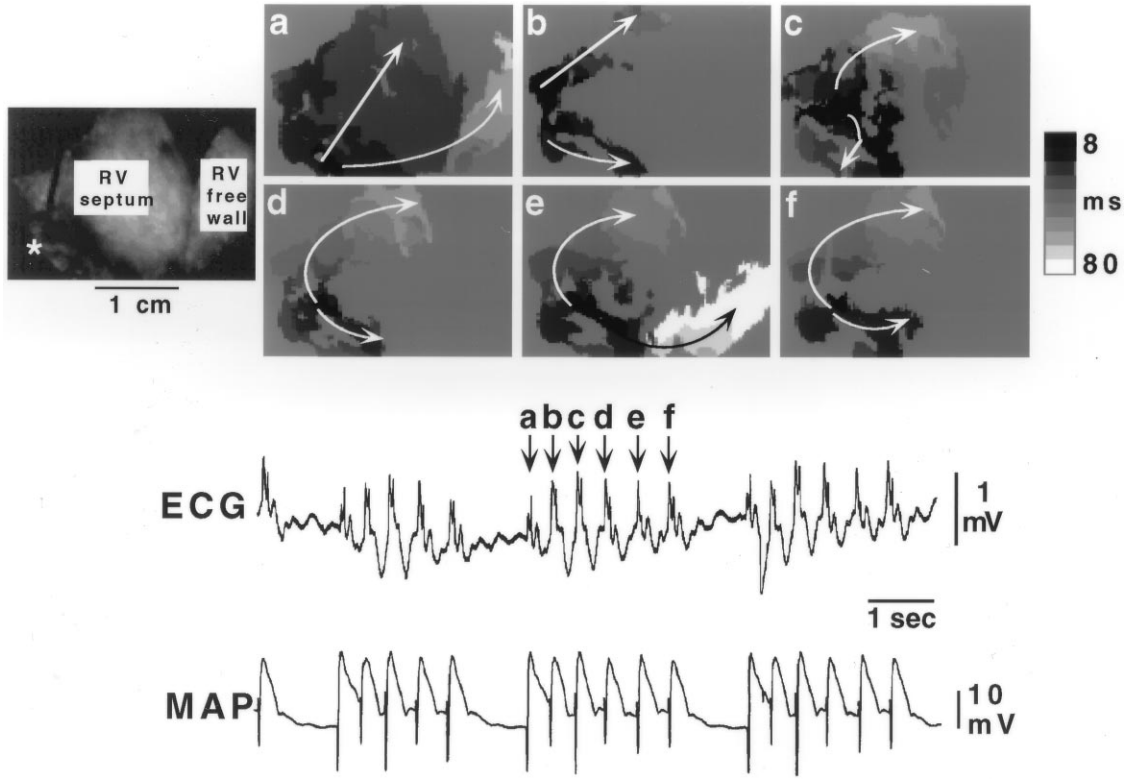

Figure 4. Endocardial maps of the RV septum and anterior free wall obtained during quinidine-induced polymorphic arrhythmia. The RV was cut open as shown in the **leftmost frame**. **Top panel** shows examples of the isochrone maps (**curved arrows** indicate direction of propagation) during an episode of polymorphic arrhythmia. Each map corresponds to the QRS deflection and MAP indicated by the respective letter shown on the ECG (**middle and bottom panels**).

charges was initiated at or near the site of recording. However, it is clear from the sequential isochrone maps of intervals a to f that the site of initial endocardial activation did change from one beat to the next over small but appreciable distances within an area confined to the lower portion of the RV septum. In addition, the very narrow areas of initial breakthrough (black) in some of the maps suggest the possibility that the site of initiation was located in the subendocardium. Moreover, the pattern of wave propagation was different for each beat. It therefore seems likely that the polymorphic ECG pattern recorded in this episode resulted from a combination of both changes in the initial site of subendocardial activation and changes in the activation sequence as a result of rate-dependent functional block.

Origin of triggered responses. In an attempt to quantify our results and determine the mechanism of the beat to beat shift of the site of origin of the triggered responses, we studied the relation between the propagation pattern of each of 157 initial ventricular discharges (whether following a spontaneous or a stimulus-induced response) and the site of origin of subsequent discharges. As illustrated in Table 1, we could divide the initial (triggering) beats into two major categories. Those that gave rise to broad wave fronts of propagation and showed no evidence of block and those in which partial block was demonstrated. Fifty-eight initial beats belonged to the first group. They propagated through the entire recording area and showed no evidence of block. Of these, 45 were followed by triggered activity, whereas 13 were not. In the former subgroup, 37 wave fronts propagated into the recording area from an unknown origin. However, eight beats were initiated from a previously activated area after partial recovery. In the second group, 99 initial discharges propagated through only part of the recording area (partial block). Of these, only 10 were not followed by triggered responses. Repetitive activity was triggered by each of 88 initial propagating waves that demonstrated partial conduction block. Ectopic beats occurring after a partially blocked wave originated in the area of block in 54 cases and in the activated area in 18 cases. In 17 cases the origin of the beat was outside the recording area and could not be determined.

From the foregoing it seems safe to suggest that 1) the vast majority of drug-induced polymorphic ventricular arrhythmias in the isolated rabbit heart preparation used here are the result of triggered activity; 2) such activity is likely to emerge from the subendocardium of the RV or LV, or both; and 3) the polymorphic ECG appearance of the arrhythmic episodes is the result of beat to beat changes of the site of origin of EADs over wide areas of the ventricular subendocardium. By providing a mechanism for transient protection of individual ectopic foci so that their triggered responses can become manifest, the occurrence of regions of transient functional block facilitates

Table 1. Origin of Triggered Activity in Relation to Propagation of Preceding Response*

Analyzed beats (157)	No evidence of block (58)	No succeeding TA (13)	
		Followed by TA (45)	Unknown origin (37)
			Originated from previously activated area (8)
	Partial block (99)	No succeeding TA (10)	
		Followed by TA (88)	Unknown origin (17)
			Originated from previously activated area (18)
			Originated from previously blocked area (54)

*Numbers in parentheses denote the number of triggered responses. TA = triggered activity.

the transfer of the origin of the triggered wave fronts from one site to another.

Functional reentry evoked by triggered activity. Previous results from several laboratories (9,10,12) have suggested that functional, stationary or drifting vortex-like reentry (spiral waves) may be initiated by the interaction of premature wave fronts with the refractory tails of waves that precede them. Moreover, we presented evidence (7,8) demonstrating that the degree of ECG polymorphism associated with spiral wave activity depends on the speed of the spiral wave drift. Thus, a stationary spiral results in monomorphic tachycardia, whereas a rapidly moving spiral can manifest on the ECG as TDP or even as ventricular fibrillation (7,8). However, from the experiments presented in the previous section, it is clear that the cycle lengths of the vast majority of polymorphic arrhythmias associated with drug-induced EADs are much longer (200 to 300 ms) and that the ECG patterns, although highly polymorphic, rarely present the typical undulating appearance that characterizes TDP. We therefore considered the possibility that under appropriate conditions, multifocal EADs may give rise to two waves that may interact such that a drifting spiral wave is initiated and that the cycle length of the reentrant arrhythmias initiated in this manner were longer in the presence of the drug. In Figure 5 we present results from an experiment in which a ventricular escape occurred after a long period of quiescence (not shown) and was followed by a run of four multifocal triggered responses. After the fourth beat, a long episode of vortex-like reentry was recorded on the epicardial surface of the ventricles. Beat to beat changes in the cycle length were observed during the first four QRS complexes, with the mean period at this time (415 ms) longer than that during vortex-like reentry (323 ms). The ECG of such an episode demonstrated a gradually undulating QRS configuration with twisting around the baseline. In the top panel of Figure 5, frames a and b show the isochrone maps of the last two triggered responses. Note that both wave fronts a and b occurred in partially refractory tissue and did not activate the entire anterior surface of the ventricles. Wave front a appeared as a breakthrough in the left ventricular apex and moved upward toward the base. Wave front b breaks through on the surface of the RV and also propagates toward the left. Immediately thereafter, another wave emerges near the apex and moves very slowly through partially refractory tissue toward the base of the LV. Eventually, the wave front is broken near the center of the field of view and begins to pivot in the counterclockwise direction around its broken end near the LAD. Thereafter, vortex-like reentry ensues, which lasts at least 24 cycles. The center of the first reentrant wave (map c) was initially located on the anterior surface of the LV but began to drift gradually upward toward the lateral LV wall (frames d to f). This gradual drift correlated with a gradual decrease in the amplitude of the QRS complex. Subsequently, although not shown by the maps, as the rotation center moved toward the posterior wall, there was a twist in the polarity of the main QRS axis, resulting in the typical appearance of TDP.

Rapid pacing-induced tachycardia in absence and presence of quinidine. In the long episode of TDP discussed in the previous section, the cycle length was somewhat shorter (323 ms) than the mean cycle length of polymorphic tachycardias resulting from multifocal EAD activation. However, the cycle length of the EAD-induced reentrant episode was still appreciably longer than the cycle lengths reported for polymorphic tachycardias initiated by burst pacing in the absence of quinidine or class III drugs. To determine whether such a difference was the result of antiarrhythmic drug effects on cycle length, we carried out additional experiments in which vortex-like reentry was initiated in the absence and presence of quinidine. As reported previously (7), in the absence of antiarrhythmic drugs, burst pacing gave rise to drifting spiral waves. The average cycle length of these episodes was 132 ± 8 ms (eight episodes). Figure 6 shows the isochrone map and ECG tracing of an episode of polymorphic tachycardia induced in this manner. Initially, the arrhythmia manifested in the continuous optical recording as a clockwise reentrant process

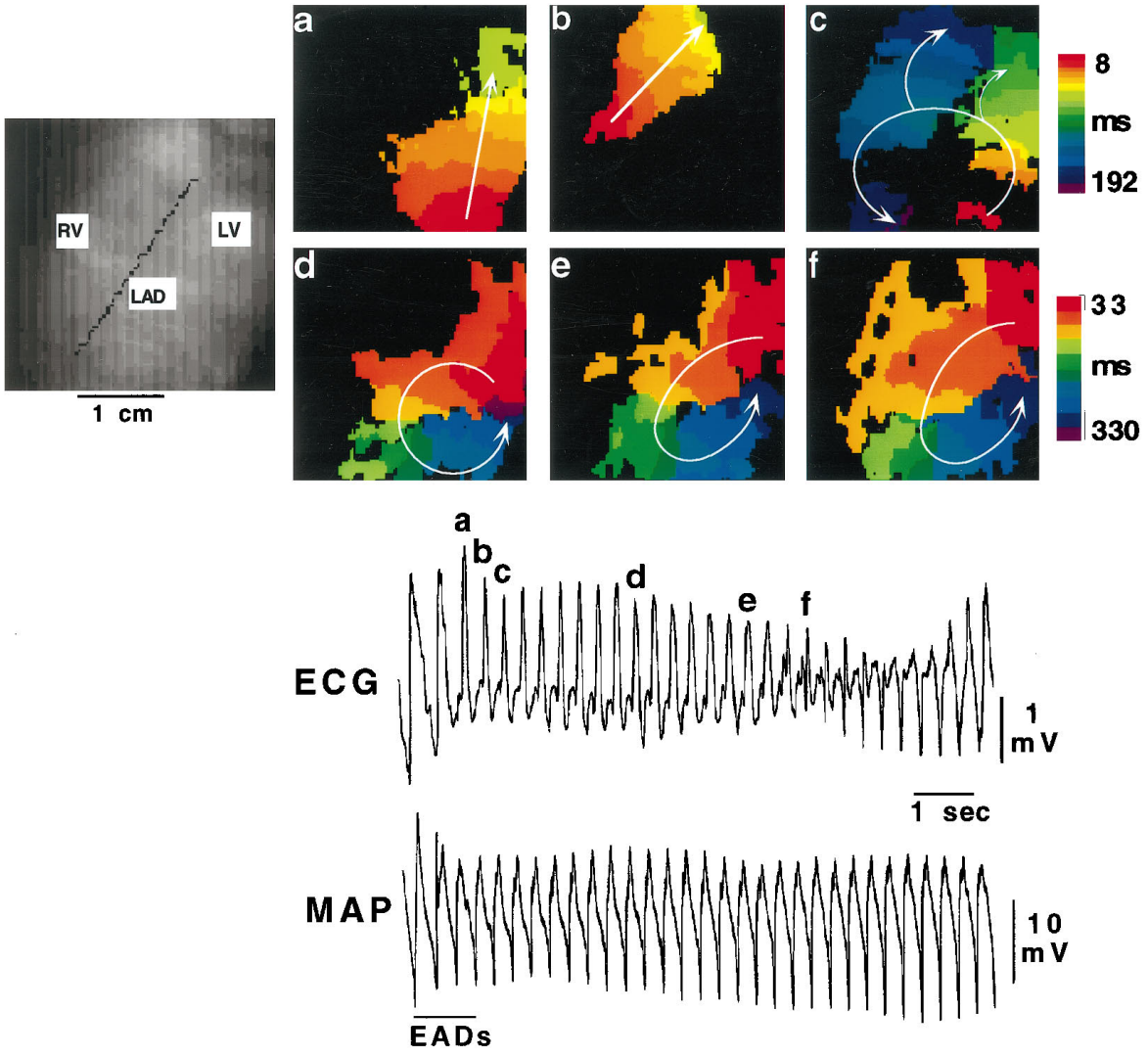

Figure 5. EAD-induced polymorphic reentrant tachycardia. **Top panel,** Examples of epicardial isochrone maps obtained from the surface of the anterior LV wall and the RV free wall during one episode in the presence of quinidine. Each map corresponds to the deflection and MAP indicated by the respective letter shown on the ECG (**middle and bottom panels**). Triggered activity (a, b and c) occurred during the period of time indicated by the **bar** below the MAP. This activity was followed by a long episode of vortex-like reentry on the epicardial surface of the ventricles (d to f). Map **c** shows the first reentrant wave. The center of the rotating activity was not stationary but gradually shifted upward and to the right. This gradual shift was associated with a gradual decrease in the amplitude of the QRS complex. Note that maps **d**, **e** and **f** are displayed with a time interval of 33 ms, although recordings were performed at a sampling rate of 120/s (~8 ms/frame).

whose center of rotation rapidly moved throughout the anterior ventricular wall (compare the position of the center of rotation in beats a to c). In subsequent beats, the rotation center frequently disappeared from the recording area (beats d and e), which resulted in the appearance of curvilinear waves that propagated from an unknown origin outside the recording field. Such dramatic changes in the position of the rotation center were accompanied by undulating changes in the QRS amplitude that resembled TDP. The cycle length of the polymorphic arrhythmia in the presence of quinidine (194 ± 12 ms, 13 episodes) was significantly longer than that of the polymorphic arrhythmia induced in the absence of quinidine ($p < 0.03$). However, the cycle length of the polymorphic tachycardia in the presence of quinidine was shorter than that of the polymorphic arrhythmias resulting from triggered activity in the absence of reentry (424 ± 19 ms, $p < 0.001$). Patterns of propagation in most of the pacing-induced polymorphic tachycardias in the presence of quinidine were similar to those observed in control conditions. Figure 7 shows an example of a drifting spiral wave obtained in the presence of quinidine. The ECG shows a gradual transition of the QRS amplitude and axis. Here, the video images taken during the initial 10 s of tachycardia showed a clockwise rotating spiral whose center of rotation was initially located near the base of the RV but then shifted slightly downward along the LAD.

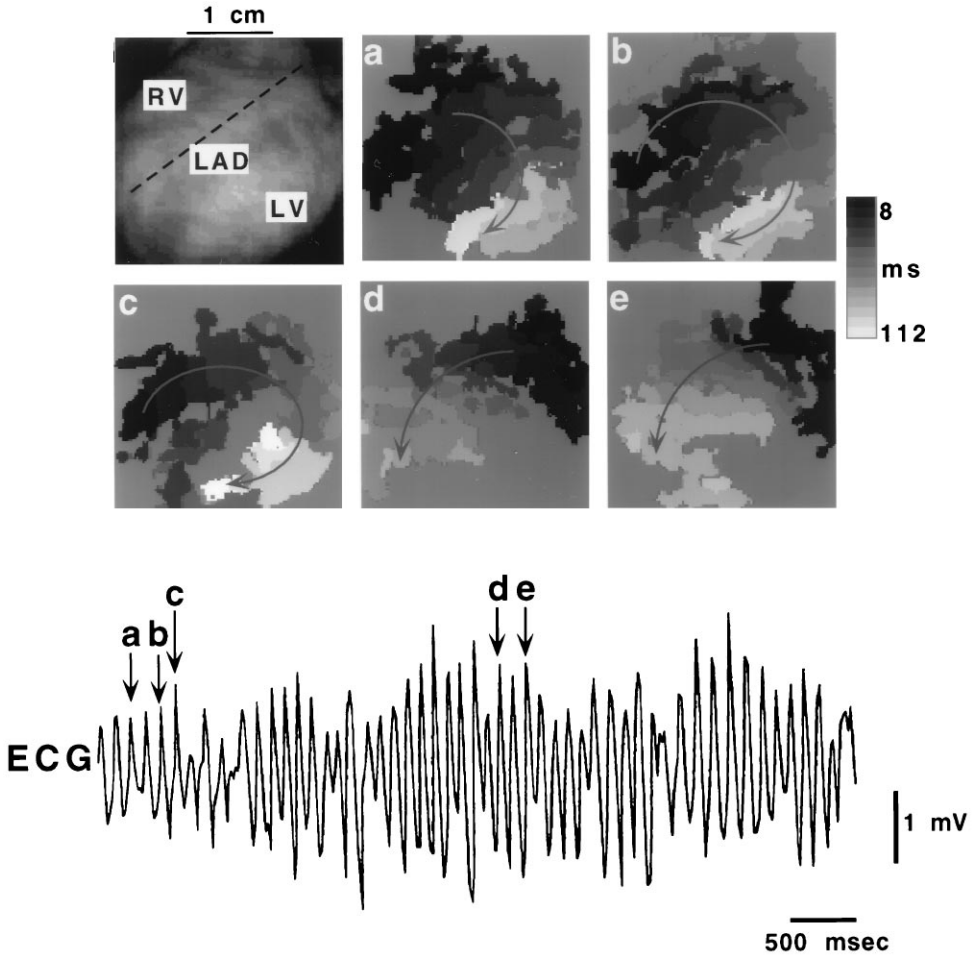

Figure 6. Top panel, Epicardial maps of reentrant polymorphic tachycardia induced by rapid ventricular stimulation in the absence of antiarrhythmic drugs. Isochrone maps of isolated beats show the image in position of the rotation center. **Bottom panel,** ECG shows a fast rate of polymorphic ventricular tachycardia.

Discussion

The most important observation in this study is that in the isolated rabbit heart, the vast majority of arrhythmia episodes observed in the presence of quinidine or E-4031 after long periods of quiescence are the result of EADs. Also, in most cases the polymorphic appearance of such arrhythmias on the ECG is the result of beat to beat changes in the site of initiation of the EADs, with consequent changes in the direction of the wave front and the activation sequence. Under appropriate conditions, multifocal EADs may occasionally set the stage for the initiation of drifting spiral wave activity, with consequent gradual undulation in the QRS configuration typical of TDP. In other words, although EADs themselves may result in a wide variety of polymorphic arrhythmia patterns as a result of beat to beat changes in the site of their initiation, they can also act as triggers for reentrant mechanisms. It is important that in the absence of antiarrhythmic agents, similar episodes of drifting spiral wave activity could only be elicited by means of burst pacing. However, the rotation period and the coupling interval of the first beat of the arrhythmia were longer in the presence of drug. Moreover, the cycle length of the arrhythmia was significantly longer in the presence than in the absence of drug. Regardless of the mechanism, to our knowledge the results provide for the first time a direct correlation between the spatiotemporal patterns of ventricular surface activation and the degree of polymorphism of the drug-induced arrhythmia on the ECG.

EADs and triggered activity. Analysis of our MAP recordings indicates that in most of our experiments, the polymorphic activation patterns in the optical mapping were the result of triggered activity in the form of EADs. However, we should note that in the absence of intracellular microelectrode recordings this assessment is not completely reliable. In fact, it has been demonstrated (13) that MAPs may show "pseudo" EADs that are not confirmed by transmembrane potential recordings. Nevertheless, the following experimental observations support the contention that EADs are responsible for the polymorphic arrhythmias in the majority of our experiments:

1. In most cases, simultaneous high resolution optical mapping showed focal activation manifested as concentric areas of subendocardial breakthrough. In general, the small size of such subendocardial breakthroughs indicated close proximity to the actual source, which did not show the expected characteristic of reentry (i.e., continuous rotating activation at the site of initiation of the arrhythmia).

2. The multifocal character of the breakthroughs also

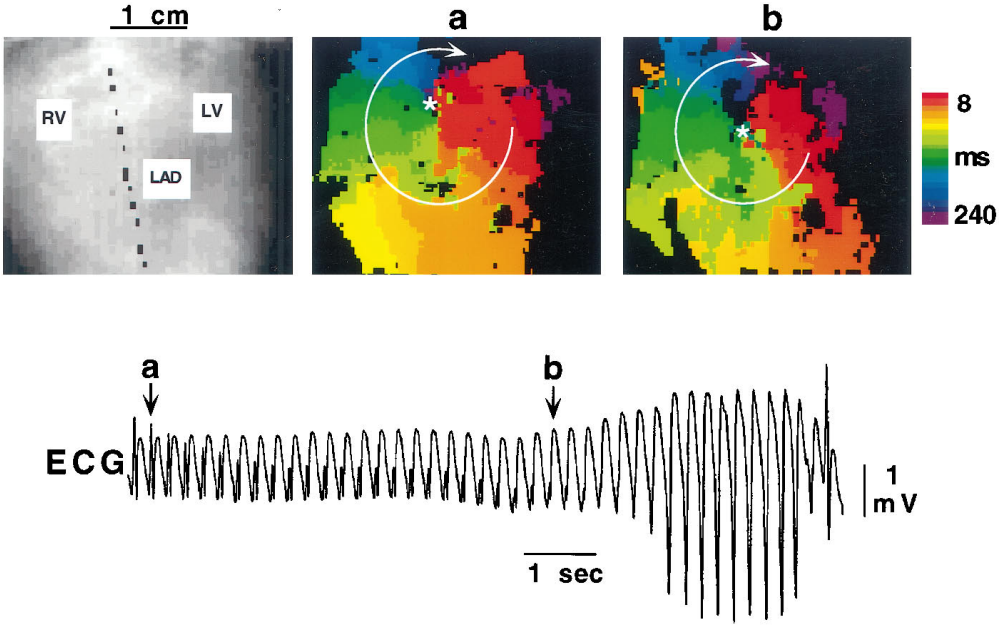

Figure 7. Top panel, Epicardial maps of reentrant polymorphic tachycardia induced by rapid ventricular stimulation in the presence of quinidine. The isochrone maps obtained during beats a and b show a clear shift in the position of the rotation center. **Bottom panel,** ECG shows a gradual transition of the QRS amplitude and axis.

cannot be fitted by any model of reentrant activity. We recently performed computer simulations using an anatomically realistic three-dimensional model of the heart and investigated the complex patterns of activation during nonstationary reentrant activation and fibrillation (7). In comparing the timing and locations of the endocardial and epicardial breakthroughs in the model with those of the experiments shown here, we found it impossible to relate the latter to a reentrant mechanism, except for those cases in which vortex-like activity was clearly manifest (Fig. 5 to 7).

3. In most episodes, the polymorphic appearance of the ECG was closely correlated with the polytopic nature of EAD-like activity, and we saw no evidence in any of our experiments of coexisting rhythmically firing automatic foci of dissimilar frequencies, which is at odds with previous speculations in published report (14).

Shifting of the site of origin during triggered activity. Triggered activity in the form of EADs may give rise to repetitive activity in a single focus. Clearly, during simultaneous repetitive firing, the activity may originate from varying sites. However, what is not clear is how the origin of individual beats may shift from one site to another. Our initial postulate was that a focus capable of undergoing EADs at a faster rate dominated during the first activation and that a second focus could gain control of the succeeding activity if it remained "protected" from the initial activation. To evaluate this possibility we correlated the origin of the triggered responses with the pattern of activation of the previous beat (Table 1). We found that in most (but not all) cases, triggered responses started from a region that was protected from previous activation. Thus, it is possible that nonuniform propagation observed during triggered activity may facilitate the shift of the origin from one site to another.

Drifting spiral wave initiated by triggered activity. Our previous results (7) demonstrated that nonstationary (i.e., drifting) spiral wave activity should give rise to patterns of TDP. However, it was uncertain whether drifting spirals could be spontaneously elicited as a result of bradycardia-induced triggered activity. In the example shown in Figure 5, a long episode of drifting spiral wave activity occurred spontaneously during coronary perfusion of quinidine. The reentrant arrhythmia developed after four consecutive focal beats that displayed varying degrees of block. The two ectopic beats that preceded the spiral activity set the conditions for the formation of a wavebreak, leading to the initiation of rotating activity. This mechanism is supported by studies in isolated slices of epicardial tissue (10,15). Starmer et al. (16) recently showed in their computer simulations of polymorphic tachycardia that a single EAD could develop into a spiral wave, giving rise to polymorphic arrhythmia. In our experiments, repetitive firing of multiple sites were responsible for maintaining heterogeneity of repolarization in the ventricles (17,18). Hence, our results clearly show that drifting vortex-like reentry may start after bradycardia-induced triggered activity. We recently demonstrated (8–10,15) that drifting reentry is always accompanied by a Doppler shift in the activation rates, which results in ECG patterns typical of TDP.

General mechanism of polymorphic arrhythmias: EADs versus reentry. Experimental evidence to date provides support for participation of EADs in polymorphic arrhythmias (4–6). In fact, all conditions known to predispose to polymorphic arrhythmias in patients are also known to induce EADs in the experimental setting (i.e., bradycardia, low potassium, low magnesium, ischemia, antiarrhythmic drugs, certain antibiotics) (9,19–22). In addition, clinical MAP recordings in patients with the acquired long QT syndrome have demonstrated the presence of EADs (23). Furthermore, agents that specifically suppress EAD-induced triggered activity are also useful in controlling TDP (e.g., magnesium, isoproterenol [24,25]). Our study provides compelling evidence that the origin and prop-

agation pattern of individual EAD-induced waves change from one beat to the next and that local activation usually occurs with varying degrees of block. In light of these mechanisms it is now possible to relate the appearance of the ECG recording to the spatiotemporal changes in the activation sequence during a wide variety of polymorphic arrhythmic patterns. However, it is still difficult to explain the gradual and predictable undulations and twisting of the QRS axis of typical TDP solely on the basis of multifocal EAD activation. It is possible that in certain cases of TDP-like activity, there is a progressive transfer of the site of origin of the EADs or a gradual shift in the excitation block pattern, which may lead to QRS complex undulations. However, in the majority of our experiments, polytopic EAD activation led to polymorphic arrhythmias whose ECG appearance was somewhat at variance with that of TDP as described by Dessertenne (2) and others (26). It was only when multifocal triggered activity established the conditions for initiation of drifting vortex-like reentry that a gradual torsion of the QRS axis was clearly manifest (Fig. 5 to 7). In addition, TDP-like arrhythmias initiated by rapid pacing were always the result of nonstationary spiral wave activity both in the presence and absence of antiarrhythmic drugs. One of the differences between episodes of EAD-induced spiral waves and spiral waves initiated after rapid pacing is that in the latter, the coupling interval of the first beat of the arrhythmia was relatively brief. This finding is consistent with the observation of Leenhardt et al. (27) in a group of patients in whom TDP occurred in the absence of prolongation of the QT interval and in whom the coupling interval of the first beat of the arrhythmia was relatively short.

Limitations of the study. Our study had some limitations that should be considered: 1) Because of the small heart size of the rabbit heart as well as potential differences in the cellular makeup of the ventricular walls, the conclusions drawn may not be completely applicable to humans. 2) The experiments were carried out in the isolated rabbit heart preparation under the artificial conditions of Langendorff perfusion of Tyrode solution. Thus, important neurohumoral factors that may have an influence on the initiation and maintenance of the arrhythmia were excluded from the study. 3) The frequencies of the arrhythmias occurring spontaneously in the presence of quinidine or E-4031 in this model are relatively slow, with cycle lengths ranging between 200 and 400 ms. Use of the term tachycardia when referring to the polymorphic ECG appearance of these arrhythmias may be called into question, particularly because the normal sinus cycle length in the rabbit heart is between 300 and 350 ms. However, the experiments were carried out under conditions of complete atrioventricular block in which idioventricular pacemaker activity at cycle lengths ≥2,000 ms usually developed. In addition, because quinidine slows conduction, and both quinidine and E-4031 prolong action potential duration even at relatively short cycle lengths, one would expect the reentrant arrhythmias to be somewhat slow. Nevertheless, we avoided the term tachycardia when referring to our experimental results. 4) Although video imaging provides unsurpassed spatial resolution of the electrical patterns on the epicardium or endocardium, or both, it does not give any information about the activity within the ventricular wall. This results in uncertainty about the precise origin of the EADs. Indeed, although the endocardial maps suggest that the activity originates near the endocardium, we cannot be absolutely sure that we can differentiate between activity originating in the subendocardial Purkinje fibers or in "M" cells in the mid-myocardium (28).

Note: While this report was being considered for publication, El-Sherif et al. (29) reported on a study of the electrophysiologic mechanisms of ventricular arrhythmias induced by the neurotoxin anthopleurin A in the dog heart. Similar to our results, their three-dimensional mapping studies suggested that the polymorphic appearance of the QRS complex during tachycardia in that model of acquired long QT syndrome was due either to focal activity that changed in origin or to the varying orientation of circulating wave fronts. Their interpretation of the latter was based on classical concepts of functional reentry occurring around long arcs of functional block. As we see it, the spatial resolution of the mapping system of El-Sherif et al. (29) was probably not sufficient to establish with accuracy the dynamics of the reentrant activity seen in their experiments. Nevertheless, we interpret their results as being compatible with the theory of wave propagation in excitable media (10,16). In fact, we propose that the reentrant activity giving rise to some of the polymorphic arrhythmias seen in such experiments may very well have been the result of three-dimensional spiral (scroll) waves drifting around the ventricles at varying speeds (7,8).

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