Catheter Ablation of Ventricular Fibrillation in Rabbit Ventricles Treated With β-Blockers

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Background—A therapeutic implication of the focal-source hypothesis of ventricular fibrillation (VF) is that VF can be terminated by focal ablation. We hypothesize that β-adrenergic receptor blockade converts multiple-wavelet VF to focal-source VF and that this focal source is located near the papillary muscle (PM).

Methods and Results—We used optical mapping techniques to study the effects of propranolol (0.3 mg/L) on VF dynamics in Langendorff-perfused rabbit hearts. The left ventricular (LV) anterior wall was mapped and optical action potential duration restitution (APDR) was determined at 25 epicardial sites. We performed ablation during VF of the left anterior PM in hearts with (N=6) or without (N=6) cytochalasin infusion, the LV lateral epicardium (Epi group, N=3), and the LV endocardium (Endo group, N=3). The PM was also ablated in 3 hearts without propranolol (control group). Propranolol converted multiple-wavelet VF to slow VF with reentry localized to the PM. Propranolol decreased the maximal slope of the APDR curve (P<0.001) as well as its spatial heterogeneity (P<0.01) and conduction velocity (P<0.01) while increasing the VF cycle length (P<0.001). PM ablation terminated VF during propranolol infusion with (6 of 6, 100%) or without (4 of 6, 67%) cytochalasin D and significantly reduced inducibility. VF did not terminate in the Epi, Endo, and control groups (P<0.001).

Conclusions—Propranolol flattens the APDR curve and reduces conduction velocity, converting multiple-wavelet VF into VF with a focal source anchored to the PM. Ablation of this focal source may terminate VF. (Circulation. 2003;108: 3149-3156.)

Key Words: ventricles ■ fibrillation ■ catheter ablation ■ mapping ■ arrhythmia

The focal-source hypothesis of cardiac fibrillation posits that fibrillation is sustained by a single high-frequency driving source. An implication of this hypothesis is that ventricular fibrillation (VF) can be terminated by eliminating this mother focus. A β-adrenergic receptor blocker is commonly used in patients at high risk of sudden death. Previous studies have shown that β-blocker therapy prolongs VF cycle length and simplifies VF activations in swine ventricles. We have shown that the papillary muscle (PM) root plays an important role in the generation of wave splitting and in the maintenance of VF. We hypothesize that a β-blocker converts multiple-wavelet VF to focal-source VF and that this focal source is anchored to the PM in isolated rabbit ventricles. We additionally hypothesize that catheter ablation of the papillary muscle may lead to VF termination in β-blocked ventricles. The purpose of this study was to test these hypotheses.

Methods

Isolated Rabbit Heart Preparation and Optical Mapping

A total of 21 New Zealand white rabbits obtained from a USDA licensed vendor in southern California (3.5 to 4.8 kg) were used in this study. The hearts were quickly removed under anesthesia, and the ascending aorta was cannulated and perfused with 37°C Tyrode’s solution equilibrated with 95% O2 and 5% CO2. The composition of Tyrode’s solution was (in mmol/L) NaCl 125, KCl 4.5, MgCl2 0.25, NaHCO3 24, NaH2PO4 1.8, CaCl2 1.8, and glucose 5.5. Coronary perfusion pressure was regulated between 80 and 95 mm Hg. A quadripolar catheter was placed on the endocardium of the right ventricle for pacing and recording (Figure 1A). For optical mapping, 5 μmol/L di-4-ANEPPS (Molecular Probes) was used as a voltage-sensitive dye. We mapped the left ventricular (LV) anterior wall (20×20 mm), acquiring 128×128 sites simultaneously at a sampling rate of 430 frames per second.

Pacing Protocol and Propranolol Infusion

We paced the right ventricle endocardium with 2-ms pulse width and twice diastolic threshold current. Effective refractory periods (ERPs) were measured by giving a premature stimulus (S2) after 8 beats (S1) at a 300-ms cycle length. Action potential duration restitution (APDR) and conduction velocity (CV) were determined using a dynamic pacing protocol. The ventricles initially were paced at a pacing cycle length (PCL) of 400 ms. After 15 to 20 stimuli had been delivered at this PCL, optical images were recorded for 1.84 seconds. The PCL was shortened progressively in increments of 20 ms for PCL >200 ms and in increments of 10 ms for PCL <200 ms until
the loss of 1:1 ventricular capture. VF was then induced by burst pacing (PCL 30 to 70 ms, 5-ms pulse duration and 5-mA current for 5 seconds). VF inducibility was determined by the ratio of successful VF induction instances to the number of burst pacing attempts (maximum 50). Once induced, VF was recorded by a bipolar electrode continuously for 15 minutes. Optical recordings were taken every 2 or 3 minutes. VF was terminated by biphasic truncated exponential waveform shocks (150 to 300 V, 6-ms total duration). After this control study, we infused 0.3 mg/L of propranolol for 20 minutes and repeated the same pacing protocols. To determine if β-blocked ventricles remained as excitable at short diastolic intervals as in the control, we constructed an APDR curve with a stimulus strength both at twice threshold and at 5 mA in 1 rabbit.

Optical Mapping and Data Analyses
Optical mapping data were processed through several image-processing algorithms. We selected 25 equally spaced epicardial points for action potential duration (APD) analysis (Figure 1B). APD was measured from phase 0% to 80% repolarization (APD80). The APD80 (Figure 1F) was plotted against its preceding diastolic interval to generate an APDR curve by an exponential fit. The spatial dispersion of the maximal slope (Smax) of APDR was determined by the standard deviation of Smax of 25 sites. We measured CV along 3 different directions (Figure 1E). The mean values of these CVs became the CV (m/sec) for that rabbit. Figure 1G shows examples of conduction time (1/CV) measurement. To calculate the phase of the optical signals, the optical action potential of each pixel during a cycle was mapped to an angular value between -π and π using a time-delay embedding method. The time delay used to analyze paced beats was equivalent to the duration of phase 0 upstroke. During VF, we measured VF cycle length and adjusted time delay to 25% of the VF cycle length. Phase maps were then constructed for the entire image sequence, and phase singularities were identified using image convolution. Phase singularity maps of accumulated phase singularities of 150 frames (345 ms) were constructed to determine the anchoring point of VF. VF was diagnosed when an irregular rhythm was present and at least 2 wavebreak points were detected within the mapped region. Irregular rhythm associated with a single wavebreak point or no wavebreak point in the mapped region is polymorphic ventricular tachycardia (VT).

Catheter Ablation
The first 12 hearts had PM ablation after propranolol infusion (Table). PM-7 through PM-12 did not receive either cytochalasin D infusion or undergo optical mapping studies. The ERP restitution curves were determined using methods reported elsewhere. Among the remaining hearts, 3 underwent PM ablation without propranolol infusion (PM-13 through PM-15), 3 were used for epicardial ablation after propranolol infusion (Epi group), and 3 were used for extensive endocardial ablation after propranolol infusion (Endo group).
To ablate the PM, we inserted a 4-mm-tip ablation catheter into the left ventricular cavity through an atriotomy. Titrated radiofrequency energy (7 to 8 W for 30 seconds, radiofrequency G-3C, Radionics Inc) or electrocautery energy (coagulation power 4 for 30 seconds, SSE-2, ValleyLab Inc) was delivered around the anterior PM base during persistent VF. In some hearts, we first cut the PM with scissors. If VF did not terminate, radiofrequency energy was delivered to the PM (Table). If VT/VF did not terminate in 10 minutes, we delivered energy one more time on the same site. If arrhythmia did not terminate, the septum near the posterior PM was ablated. After the experiments, the LV was opened and the ablation site was documented (Figure 2A). In the Epi group, we administered the same ablation energy on the LV epicardium to reduce cardiac mass to the same extent as in PM ablation (Figure 2B). In the Endo group, radiofrequency energy (7 to 8 W) was delivered for 3 minutes to the LV endocardium, including the septum (Figure 2C). All ablation procedures were performed during VF that persisted for more than 5 minutes. In case of VF termination, we attempted to reinduce VF by burst pacing. In 2 rabbit hearts, we used a LASER optical fiber to illuminate the LV chamber from the endocardium (Figure 2D). The PM base was identified as an indentation by its shadow. A needle was pushed from the epicardial side (Figure 2D) to identify the location of that shadow. When we opened the LV, the location of the pin was at the PM root (arrow, Figure 2E).

Statistical Analysis
Data are presented as mean±SD. Paired t tests were used for statistical comparisons of electrophysiologic parameters. To compare the VF inducibility and defibrillation rate of each group, Fisher’s exact test and ANOVA Newman-Keuls tests were used, respectively. The null hypothesis was rejected at a value of P≤0.05.

### Results

#### PM Ablation Terminates Slow VF and Decreases VF Inducibility
Propranolol prolonged VF cycle length and converted fast VF into slow VF or polymorphic VT. VF terminated either during or within 7 minutes (median, 44 seconds) after ablation in all 6 ventricles with cytochalasin D infusion (100%. Figures 3A and 3B) in rabbits PM-1 through PM-6. In contrast, no defibrillation occurred in the Epi or Endo or in 3 hearts without propranolol infusion (PM-13 through PM-15) (0 of 9, 0%, P=0.001). The lesion size created in the PM group (201.0±66.5 mm³) did not differ significantly with the lesion size in the Epi group (273.7±91.3 mm³, P=NS). By experimental design, lesion size in the Endo group was much larger (609.0±326.7 mm³). In PM-4 through PM-6, the resection of the anterior PM with scissors failed to terminate VF. We then delivered radiofrequency energy and successfully terminated VF. In PM-3, slow VF converted to VT as soon as we grasped the PM with forceps. VF was then terminated during ablation (Figure 3B). During propranolol infusion, the preablation VF inducibility in the PM-1 through PM-6 group was 16.2%. The inducibility decreased to 3.2% after ablation (P<0.01). Among 21 episodes of slow VT termination in the PM group, 83.3% changed to transient slow VF and then terminated (Figure 3C).
Effects of Propranolol on VF
VF was successfully induced at baseline burst pacing in all rabbits. The ratio between successful induction and the number of attempts (VF inducibility) was 44.7%. The VF cycle length (VFCL) at baseline was short. There was a highly variable morphology with fractionated, low-amplitude, or oscillatory electrogram potentials (Figure 4A). Propranolol lengthened VFCL, and electrograms became more regular (Figures 4B and 4C). The average VFCL during propranolol infusion was significantly longer (114.5 ± 25.6...
ms) than that at baseline (88.2±12.8 ms, P<0.001; Figure 4). Epicardial dominant frequency distribution (Figures 2F and 2G) and VFCL distribution (Figures 2H and 2I) showed that propranolol reduced the VF activation rate. Intermittent rapid and fractionated activities were observed both over the PM (Figure 4A) and away from the PM (Figure 4B). The voltage maps and phase maps before and after propranolol were distinctly different. Wave-wave interaction, wavebreak, and the generation of new reentry occurred continuously in baseline VF (Figure 4A). Multiple wavelets (>3) and short-lived phase singularities were scattered all over the mapped field. The corresponding S_{max} of APDR during paced rhythm was always greater than 1. After propranolol, wave-wave interactions and spontaneous wavebreak rarely occurred on the epicardium, and the electrograms showed either slow VF or polymorphic VT. The mapped area showed the presence of a single, organized, long wavefront or periodic epicardial breakthrough patterns. The number of wavelets was less than 3, and the sites of breakthrough or phase singularities localized near the PM area drove reentry with a large core size (Figure 4B). The corresponding S_{max} of APDR during paced rhythm was less than 1. With a higher concentration of propranolol (0.6 mg/L) in a separate study, slow VF was maintained with figure-eight reentry anchored on the PM or an epicardial coronary artery (Figure 4C). At that time, phase singularities were scattered along the anchoring points of figure-eight reentry. Three ventricles developed transient VT after ablation (Table).

**Propranolol Decreases APDR and Excitability**

Propranolol prolonged AP_{D50} significantly with PCL of 300 ms (from 161.8±12.2 to 176.6±13.5 ms, P<0.01; Figures 5A and 6A). Not only did propranolol decrease the S_{max} of APDR (from 1.13±0.19 to 0.79±0.26, P<0.001; Figures 5A and 6B), it also decreased the spatial dispersion of S_{max} of APDR (from 0.13±0.05 to 0.08±0.03, P<0.01; Figure 6C). The shortest diastolic interval reached during propranolol was longer than that at the baseline. This is true even when the pacing output was increased to 5 mA (16.7 times of pacing threshold), indicating reduced excitability (Figure 5B). Propranolol also increased the pacing threshold (0.44±0.24 versus 0.84±0.35 mA, P<0.01) and ERP (155.9±17.1 versus 191.8±24.4 ms, P<0.0001, Fig-

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**Figure 4.** Voltage maps (upper row), phase maps (lower row), and phase singularity maps (PS) during baseline VF (A), slow VF with 0.3 mg/L propranolol (B), and polymorphic VT with 0.6 mg/L propranolol (C), respectively. Left column shows the optical action potentials taken from sites a and b on the voltage maps. A simultaneous bipolar recording is also shown (bipolar EGM). White blue dots on PS maps correspond to epicardial locations of PM. White arrowhead on phase singularity map of (C) indicates a secondary branching point of obtuse marginal branch of circumflex coronary artery, and the dotted square is the mapped area. Coronary artery is delineated by white lines. The numbers on the right lower edges of each map are times (ms) of activation, with the beginning of the data collection as time zero. See details in the text.
Average CVs at the PCL 300 ms were significantly lower during propranolol infusion (68.4 ± 14.9 m/s) than at the baseline (84.0 ± 8.1 m/s, \( P < 0.001 \)). Propranolol slowed conduction around the PM area. PM ablation additionally reduced CV (Figures 1D and 1E).

**Studies Without Cytochalasin D**

In rabbits 7 through 12, we gave propranolol but omitted cytochalasin D. PM ablation terminated VF in 4 of the 6 hearts. In the remaining 2 rabbits (PM-8 and PM-12), VF was terminated by ablating the posterior PM. The electrophysiologic parameters without Cyto-D were consistent with the data with Cyto-D. ERPs measured by S1S2 extrastimuli were prolonged after propranolol (137.0 ± 1.41 versus 153.0 ± 4.2 ms, \( P < 0.01 \)). Propranolol flattened the \( S_{max} \) of ERP restitution curve (0.31 ± 0.21) compared with baseline (0.86 ± 0.49, \( P = 0.038 \)). It also prolonged the VFCL from 64.5 ± 1.5 (baseline) to 107.7 ± 4.9 ms.

**Figure 5.** A, Restitution curves taken from 25 epicardial points on the mapped area (Figure 1B). \( \bullet \), APD\(_{80} \) at baseline; \( \circ \), APD\(_{80} \) during propranolol infusion. The numbers above and below the APDR restitution curves are maximum slopes (\( S_{max} \)) during propranolol infusion and at baseline, respectively. B, Restitution curves obtained with dynamic pacing protocol at baseline (\( \bullet \)) and during propranolol infusion (\( \circ \)). The stimulus strength was twice threshold at baseline and was 5 mA (16.7 times the threshold) with propranolol. Even though the pacing output was much stronger, the ERP during propranolol infusion was still longer than baseline and that of the slope of restitution was flatter than baseline.
Discussion

In this study, we demonstrated that propranolol reduced $S_{\text{max}}$ of the APDR and reduced excitability, converting multiple-wavelet VF into a slower focal-source VF with limited reentrant wavefronts anchored to specific anatomical structures, such as the PM. Ablation of the PM resulted in successful termination of VF, suggesting that the PM-anchored wavefront maintained the VF.

Roles of APDR and Excitability in VF Maintenance

Wu et al. reported that both APDR and excitability are important in VF maintenance. Steep APDR and normal excitability favor the development of multiple wavelet, whereas flat APDR and depressed excitability favor a focal-source mechanism. Propranolol is known to significantly reduce VF cycle length, decrease the excitability, and flatten the slope of APDR. These effects converted multiple-wavelet VF into focal-source VF. Elimination of the focal source by radiofrequency ablation resulted in VF termination.

Importance of Structural Heterogeneity in the Maintenance of VF

The results of this study highlight the importance of structural heterogeneity in the maintenance of VF when APDR is flattened and excitability is reduced. Previous studies show that both the preexisting structural heterogeneity and the APDR are important in the mechanisms of wavebreak. Kim et al. reported that the geometry of the ventricular wall and anisotropic anatomic structures like the PM root play a role in wave splitting and the maintenance of VF. Abrupt change of fiber orientation or a specific spatial distribution of the Purkinje-muscle junction at the PM base results in a conduction delay and reentry. Conduction delay around the PM might also account for human VF in some cases. Our present study demonstrates that such a mechanism could account for VF in rabbit ventricles treated with $\beta$-blockers. However, the rabbit heart is much smaller than the human heart. Whether the results of the present study are applicable to human patients is unclear.

Figure 6. A, Electrophysiologic parameters at baseline and during propranolol infusion. B, $S_{\text{max}}$ of APDR was reduced by propranolol in all rabbits. C, Reduced spatial dispersion of $S_{\text{max}}$ of APDR (SD APDR) by propranolol was observed in all but 1 heart.

Clinical Implications

Because of poor coupling between Purkinje fibers and the ventricular myocardium at the root of the PM, the best place to register discrete Purkinje potential in the left ventricular endocardium was at or near the PM. We hypothesized that verapamil-sensitive idiopathic VT in humans is attributable to a reentrant wavefront that anchors to the PM. Catheter ablation guided by the Purkinje potential might eliminate this VT. Haissaguerre et al. reported that Purkinje potentials are also present at the source of human idiopathic VF. Because the endocardial Purkinje potentials normally colocalize with the PM, these findings suggest that reentry anchored near the PM might also account for human VF in some cases. Our present study demonstrates that such a mechanism could account for VF in rabbit ventricles treated with $\beta$-blockers. However, the rabbit heart is much smaller than the human heart. Whether the results of the present study are applicable to human patients is unclear.

Limitations

One limitation of the present study is that we did not map the entire heart. In addition, the contribution of transmural or endocardial activations is unclear because of the limitation of epicardial mapping.

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