Destabilization and Early Termination of Spiral-Wave Reentry by a Class III Antiarrhythmic Agent, Nifekalant, in a Perfused Two-Dimensional Layer of Rabbit Ventricular Myocardium

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Abstract—Nifekalant (NF) is a novel class III antiarrhythmic agent that is effective in preventing recurrent life-threatening ventricular tachycardia/fibrillation (VT/VF). However, because the mechanism underlying the antiarrhythmic action of NF is unknown, we investigated the effects of NF on the dynamics of functional reentry in rabbit hearts. A 2D subepicardial myocardial layer was prepared in 21 Langendorff-perfused rabbit hearts by cryoablation of the left ventricular cavity. Action potential signals were recorded and analyzed by a high-resolution optical mapping system. During basic stimulation, NF (0.1 μmol/L) significantly prolonged action potential duration without affecting conduction velocity. VTs induced by DC stimulation in the presence of NF had a longer cycle length (188±31 ms, NF, versus 154±16 ms, control, P<0.05) and were of shorter duration (VTs>30 s: 2/54, NF, versus 19/93, control). Spiral-wave excitations after NF were characterized by a greater meandering with longer lines of functional block and more frequent wavefront–tail interactions, resulting in either breakup of the spiral-wave or a sudden spatial jump of the phase singularity. In controls, rotors terminated spontaneously, mostly as a result of mutual annihilation of counter-rotating wavefronts. With NF, rotors frequently terminated by wavefront collision with the atrioventricular groove or by trapping the spiral tip in a refractory zone. This modification was the result of the limited proportion of excitable tissue in the ventricles. NF destabilizes spiral-wave reentry in the heart through modulation of repolarization, leading to early termination or breakup. (Circ Res. 2006;98:0-0.)

Key Words: spiral-wave reentry ■ IKr blocker ■ nifekalant ■ optical mapping ■ ventricular tachycardia

Spiral-wave reentrant excitations rotating around a functional obstacle are the major organization center of life-threatening ventricular tachycardia and fibrillation (VT/VF).1,2 Pharmacological regulation of such rotors is therefore the central task to be achieved for efficient prevention of sudden arrhythmic death.3,4 Nifekalant hydrochloride (NF) is a new class III antiarrhythmic drug developed in Japan that causes dose-dependent prolongation of action potential duration (APD) in both atrial and ventricular muscle, mainly by reducing the rapid component of the delayed rectifier K+ current (IKr),5–9 and at higher concentrations, NF has an inhibitory effect on other voltage- and ligand-gated K+ currents.5–8 APD prolongation by pharmacological blockade of IKr renders a certain proarrhythmic propensity which is known as drug-induced QT prolongation and torsades de points (TdP), and this potential risk limits the use of class III antiarrhythmic drugs.10 In experimental animals, however, NF has been shown to prevent VT/VF after acute myocardial infarction without compromising hemodynamics11 and improve electrical defibrillation efficacy.12 Several clinical studies have shown the usefulness of intravenous NF in the treatment of patients with recurrent VT/VF that is resistant to other antiarrhythmic drugs and DC shocks.13,14 It seems reasonable to hypothesize that this unique antiarrhythmic potential of NF is mediated by a modulation of spiral-wave dynamics in the heart, and the present study was designed to test this hypothesis in a perfused 2D layer of rabbit ventricle by using our custom-made, high-resolution optical mapping system.

Materials and Methods

Langendorff-Perfused Rabbit Heart and Optical Mapping

Isolated rabbit hearts were continuously perfused on a Langendorff apparatus with modified Krebs Ringer solution equilibrated with...
95% O₂/5% CO₂ to maintain a pH at 7.4 (37°C). Complete atrioventricular block was produced by ligation of His bundle. We created a 2D epicardial layer of ventricular myocardium (1.0±0.2 mm thick) by cryoprocedure (Figure 1A). This model is similar to that reported by Schalij et al.15 The tissues were stained with a voltage-sensitive dye, di-4-ANEPPS. To minimize motion artifacts, 15 mmol/L 2,3-butanedione monoxime (BDM) was added, unless otherwise specified. The heart was illuminated with bluish light-emitting diodes (LEDs); the emitted fluorescence was recorded with a solid-state image-sensing digital video camera (Fastcam-Ultima 40K, Photron) to acquire the image at 750 frames/s. To reveal the signal, the background fluorescence was subtracted from each frame and low-pass spatial filtering was applied. The wavefront–tail dynamics during VT was visualized by connecting the 10% depolarization points in the action potential upstrokes as the wavefront and by connecting 90% repolarization points as the wave tail. The pattern of wave propagation during VT was quantified using phase mapping method.16 More details regarding the experimental model and procedures of optical mapping are described in the online data supplement available at http://circres.ahajournals.org.

Experimental Protocols

Conduction velocity and APD at 90% repolarization (APD₉₀) were measured during constant (S1) stimulation at the center of the left ventricular free wall at basic cycle lengths (BCLs) of 180 to 800 ms. We used the dynamic pacing method17 to characterize the APD restitution property. In the experiments to induce VT, 18 S1 stimuli at a BCL of 400 ms were applied to the apex, and a 10-ms DC stimulus (S2, monophasic pulse at 20 V) was delivered through Ag-AgCl paddle electrodes placed on the lateral surface of both ventricles (modified cross-field stimulation) during the vulnerable window of the final S1 excitation (Figure 1B). Widely spaced bipolar electrograms were recorded between the apex of the left ventricle and the high lateral wall of the right ventricle to monitor the whole ventricular excitation. Data were obtained before (baseline) and 20 to 30 minutes after application of 0.1 μmol/L nifekalant (Nihon Schering, Japan). More details regarding the experimental protocols are described in the online data supplement.

Statistical Analysis

Group data were expressed as means±SD. Statistical comparisons were performed by 2-way analysis of variance (ANOVA) with Tukey, t test, or Mann–Whitney U test when appropriate. Differences were considered significant when the probability value was <0.05.

Results

Conduction Velocity and Action Potential Duration

Conduction properties and action potential configurations were examined in seven hearts during constant stimulation. The isochrones of activation front exhibited a smooth, symmetric, elliptical pattern (Figure 1C); the long axis of the ellipse corresponded to the fiber orientation of subepicardial cardiac muscle.15 In the central 18×18 mm square, there was a linear correlation between activation times and distances in both the longitudinal direction (LD) and the transverse direction (TD). Conduction velocity in the LD and TD at a BCL 180 to 800 ms before (control) and after application of 0.1 μmol/L nifekalant (NF) (means±SD, n=7).

Figure 1. Rabbit ventricular tissue preparation and its anisotropic conduction property. A, Two-dimensional subepicardial myocardial layer that survived after endocardial cryoablation (a section of a heart stained red with 2,3,5-triphenyltetrazolium chloride). B, Anterior view of a rabbit heart after cryoablation (Langendorff perfusion for optical mapping). DC indicates paddle electrodes for DC stimulation; BE, a pair of electrodes for bipolar electrograms. C, Isochrone map of activation (2.67-ms intervals) during constant stimulation (BCL 800 ms). The dotted square surrounds the area for measurements of conduction velocity and APD. LD indicates longitudinal direction; TD, transverse direction. D, Conduction velocity in LD and TD at a BCL 180 to 800 ms before (control) and after application of 0.1 μmol/L nifekalant (NF) (means±SD, n=7).
Figure 2A shows representative changes in APD in response to NF application. Action potential signals recorded at 16 sites covering the 18×18 mm square were superimposed. NF (0.1 μmol/L) caused uniform prolongation of APD at all recording sites. Figure 2B summarizes the changes in APD90 (average of the values at the 16 sites) after application of NF. NF caused significant prolongation of APD90, and the prolongation was greater at longer BCLs. The dispersion of the APD90 values among the 16 recording sites was virtually unaffected by NF at BLCs ranging from 180 to 800 ms (Figure 2C).

Figure 2D and E shows representative data for the APD restitution. The plots of APD90 versus the diastolic intervals before and after NF (0.1 μmol/L) both fitted single exponential functions well (Figure 2E). The maximal slope after NF (0.90) was much greater than under the control conditions (0.55). The average of the maximum slope values in 5 hearts increased from 0.48±0.20 in control to 0.70±0.24 after NF (n=5, P<0.05). NF also enhanced the APD alternans at shorter BCLs; the maximum alternans amplitude was increased from 5.8±2.2 ms in control to 12.5±5.5 ms after NF (n=5, P<0.05).

The NF-induced changes in action potential configuration were not attributable to time-dependent deterioration of the 2D tissue preparation, because we confirmed in pilot experiments that the conduction velocity, APD90, and the APD restitution were unchanged for 120 minutes of Langendorff perfusion (online data supplement).

**VT Induced by Modified Cross-Field Stimulation**

VTs were induced in 15 hearts by DC stimulation (S2) applied during the vulnerable window of basic (S1) excitation from the apex. Under drug-free conditions (control), 93 VTs were induced in an S1-S2 interval of 140 to 230 ms. The VTs were polymorphic during the first several beats, but became more stable during the subsequent beats and had almost a monomorphic configuration. VT cycle length (VTCL) averaged 154±16 ms. Of the 93 VTs, 74 (79.6%) terminated
spontaneously within 30 s (nonsustained), whereas the other 19 (20.4%) persisted for more than 30 s (sustained). Forty-six of the 52 nonsustained VTs terminated within 5 s (Table). After application of NF (0.1 μmol/L), VTs were induced with longer S1-S2 intervals (150 to 270 ms), and they were always polymorphic and terminated earlier than under control conditions. Of the 54 VTs in the presence of NF, 52 VTs (96.2%) were nonsustained, and the other 2 (3.7%) were sustained. Forty-four of the 52 nonsustained VTs terminated within 5 s (Table). VTCL was significantly longer after NF (188 ± 31 ms) than under control conditions (P < 0.05).

Dynamics of Spiral-Wave Excitation

Video images of excitation during VT were analyzed in the 9 hearts. Under the control conditions, certain forms of rotors (single-loop or figure-of-eight reentry) were documented in 37 (61.7%) of the 64 VT episodes. The remaining 27 VT episodes showed 1-way propagation of wavefronts traversing the observation area.

Figure 3A shows representative experiments in a heart under control conditions (supplemental Movie I). Isochrone maps of 4 cycles, 2 s after VT initiation, are shown (top panels) together with bipolar electrograms. Clockwise rotation of wavefronts around a line of block (4.8 to 7.0 mm) with a VTCL of 136 to 141 ms can be seen. The line of block, which was roughly parallel to fiber orientation in this VT episode (Figure 3B), was concluded to be functional, because there was no appreciable conduction delay in the area during constant stimulation. The line also moved to other sites during different VT episodes in the same heart (Figure 3C). The circuit was more or less stable for more than 10 s and exhibited moderate meandering. Optical action potential signals recorded around the circuit are shown in Figure 3B. Action potentials at sites close to the pivot points were characterized by a longer time to peak (slower upstroke at sites b and d). After passing through the pivot point the action potential had a shorter time to peak (faster upstroke at sites a and c). The action potentials recorded at the center of the functional block line exhibited low-amplitude double potentials (site e). There were no isoelectric segments, which may reflect electrical diastole, between successive action potentials throughout the circuit. Figure 3C shows the lines of the functional blocks observed during 5 VTs in 4 hearts under the control conditions (4 rotations are superimposed in each VT episode). The lines run either parallel (≈70%) or across (≈30%) the fiber orientation. The lines were often alongside of epicardial blood vessels. There was no obvious tissue damage or macroscopic structural discontinuity to anchor the reentrant pathway in a fixed position.

Figure 4A shows activation patterns during a short VT (lasting for 3 s) induced after application of NF (0.1 μmol/L) (supplemental Movie II). Rotors circulating around functional block line(s) were recognized in isochrone maps during the 4 consecutive cycles, but their circuits changed dramatically in a beat-to-beat manner, with VTCLs varying from 169 to 190 ms. In beat 1, after turning around the left end (site d) of a long (>12 mm) functional block line the wavefront faced its opposite side (site c), giving rise to a further extension of the circuit. In beat 2, the clockwise rotation was maintained around more complex y-shaped lines of block. In beat 3, double circuits of clockwise rotation were seen around 2 functional block lines, and the total length of the 2 lines reached 12.3 mm. In beat 4, the wavefronts exhibited clockwise rotation with a more complex trajectory around an extremely long (21.1 mm) and tortuous line of function block. The rotor terminated spontaneously 3 cycles later (not shown). Bipolar electrogram during the VT episode showed polymorphic TdP-like ventricular excitations. The action potentials recorded from the circuits (Figure 4B) showed marked beat-to-beat variation. The action potentials took a longer time to peak at sites close to the pivot points (site d in the first cycle and site a in the second cycle). Clear low-amplitude double potentials were recorded on the functional block line (site c in the first cycle).

Qualitatively similar results were obtained in all 9 hearts exhibiting visible rotors under the control conditions as well as after NF. Figure 4C shows the lines of functional block observed during VTs in 4 hearts after NF (4 VT cycles are superimposed in each heart). Thus, application of NF caused marked prolongation of the functional block line, considerable meandering of the circuit in association with frequent local conduction block, and earlier termination of the rotation.

Wavefront–Tail Interaction and Phase Singularity

The modification of the spiral reentry dynamics by NF was investigated more extensively in terms of wavefront–tail interaction and phase singularity. Every 7 VT episodes with rotors visible in the observation area before (control) and after application of NF were analyzed by constructing wavefront–tail and phase maps. During VTs under the control conditions, the wavefront chased its own tail with a certain distance between them (repolarized zone), and the wavefronts seldom met each other (Figure 5A, top). The number of phase singularity points (PSs) in the phase maps was normally (>90%) 1 (a single rotor) (Figure 5A, bottom) and, rarely, increased to 2 for short periods (Figure 5D). In the presence of NF (0.1 μmol/L), the wavefront frequently encountered with its own tail, causing transient breakup of the spiral-wave (Figure 5B, top) or sudden movement of the organization center of rotation to another site (Figure 5C, top). In the phase maps, the former was recognized as an increase in PS from 1 to 3 (Figure 5B, bottom), whereas the latter was recognized as
a sudden jump in PS site (Figure 5C, bottom). Figure 5D shows representative changes in PS numbers/500 frames (665 ms) after application of NF. Pooled data are summarized in Figure 5E; the average number of PSs during 500 frames (665 ms) was 1.13 ± 0.14 in the control and 1.63 ± 0.22 after NF (n=7, P<0.05).

Mode of Spiral-Wave Termination
The mode of spontaneous termination of spiral-wave excitation was analyzed in 10 VT episodes in the absence of NF (control) and 19 VT episodes in the presence of NF. In the controls, most VTs (9/10, 90.0%) terminated as a result of mutual annihilation of counter-rotating spiral-waves. Figure 6A shows a representative experiment (supplemental Movie III). Excitation patterns for the final beat of a VT episode are shown in 4 sequential phase maps (left). A pair of PSs with opposite chiralities constructing a figure-of-eight reentry circuit was present in the lower region of the left ventricle (1850 ms). The distance between the 2 PSs initially increased (1850 to 1935 ms) and then decreased (1935 to 1951 ms), culminating in mutual annihilation (1961 ms). The trajectories of the 2 PSs plotted on space (x, y) and time axes are shown in the middle (red, clockwise; blue, counterclockwise). Action potential signals recorded from 6 sites in the figure-of-eight reentry circuit (right) revealed conduction block at the central common pathway (site d). In the remaining control episode, the VT ended by extinction of a single rotor when it collided against the anatomic boundary (atrophicentric groove).

In the presence of NF, 12/19 VTs (63.2%) terminated by rotor extinction after considerable meandering toward the anatomic boundary. Figure 6B shows an example (supple-
In the 4 sequential phase maps (left), a clockwise rotating PS initially moved a long distance from the right upper region to the right margin, then back toward the middle upper region (1282 to 1382 ms), and was finally pushed out of the atrioventricular groove (1463 to 1490 ms). The trajectory of the PS plotted on space and time axes is shown in the middle (the blue wall on the right indicates the atrioventricular groove), and action potential signals recorded from 5 sites in the meandering pathway are shown at the right.

In the remaining 7 VTs (36.8%) in the presence of NF, the rotors terminated by trapping the spiral tip in a region entirely surrounded by refractory tissue. Figure 7 shows a representative experiment. Isochrone and APD$_{90}$ maps of 3 beats before termination are shown in Figure 7A. In beat 1, activation from the left upper region turned around a short functional block line (yellow) in a clockwise direction. In beat 2, the clockwise rotation was maintained and associated with extension of the functional block line toward the central region. In beat 3, the wavefront from the left upper region was blocked in the central region. Because this wavefront (red dotted line) was entirely surrounded by a refractory zone, no offspring wavelets emerged, and the VT terminated. Action potential signal tracings from 2 sites (asterisks) revealed APD alternans during the VT cycles (Figure 7B), with the longest APD$_{90}$ of beat 3 (site a) preventing further rotation of the wavefront. The APD$_{90}$ maps of beat 3 visualized trapping of the entire spiral tip by the long APD$_{90}$ zone. A similar enhancement of APD alternans preceding the spontaneous termination was observed in all the 7 VTs of this group. Figure 7C shows phase maps of the final beat (supplemental Movie V). The PS of clockwise rotation shifted from the left upper region to the right margin, then back toward the middle upper region.
upper region to the center and then disappeared. The trajectory of the PS plotted on space and time axes is shown in Figure 7D.

VT Induced in the Absence of BDM
In 3 hearts, VTs were induced in the absence of BDM. The results were essentially similar to those obtained in the presence of BDM. VTs after NF (0.1 μmol/L nifekalant (NF), causing a transient breakup of a rotor. C, Interaction of a wavefront with a wave tail during VT after NF, causing a sudden movement of the organizing center to another site. PSs are indicated by circles (black for clockwise rotation, and white for counterclockwise rotation). D, Number of PS over 500 frames (665 ms) during VTs before (top) and after (bottom) NF (from the same heart as in A through C). E, Average number of PS/500 frames (665 ms) before (control) and after NF (means±SD, n=7, *P<0.05 vs control).

Discussion
The results of this study have revealed characteristic effects of NF on the spiral-wave reentry (VT) induced in isolated rabbit hearts having a 2D myocardial structure and uniform anisotropic conduction properties. NF increased the VTCL and caused its early termination because of marked destabilization of the spiral dynamics (considerable meandering and frequent wavefront–tail interactions).

Action Potential and Conduction Properties
In this study, we used an NF concentration of 0.1 μmol/L, which roughly corresponds to the plasma concentration in humans following intravenous NF administration (0.2 to 0.5 mg/kg). At this concentration, NF was shown to suppress primarily IKr in ventricular myocytes. NF has an inhibitory effect on other potassium currents but only at much higher concentrations (≥3 μmol/L). NF caused spatially uniform APD prolongation during constant stimulation in our rabbit ventricular muscle preparation, whereas conduction velocities were unaffected. The APD prolongation was significant even at a BCL of 180 ms, but it was greater at longer BCLs (400 to 800 ms). NF increased the slope of the APD restitution curve, and the change was associated with an increase in APD alternans at shorter cycle lengths. All of these NF-induced alterations in the steady-state and dynamic electrophysiological properties of ventricular muscle can be ascribed to IKr blockade. In action potential clamp experiments, Hua and Gilmour recently demonstrated that IKr contributes importantly to ventricular muscle repolarization during normal and high-
frequency stimulation and that APD alternans is regulated substantially by time- and voltage-dependent activation and deactivation of I_{Kr}.

**VTs of Spiral-Wave Reentry**

Our optical mapping analysis showed that spiral-wave excitations circulating around functional block lines were induced in the observation area in more than half (61.7%) of all VT episodes under the control conditions. The VTs induced were polymorphic during the initial several beats, but became almost monomorphic during subsequent beats. This transition is characteristic of reentrant VTs induced in a 2D myocardial layer with uniform anisotropy. Microscopic structural discontinuities in association with anisotropic fiber orientation or epicardial blood vessels may provide a basis for this anchoring behavior.

VTs induced in the presence of NF are characterized by longer cycle length, polymorphic configuration, and earlier termination than those observed in the controls. The incidence of spontaneous termination within 5 s was doubled after NF (Table). This indicates that I_{Kr} blockade affects the evolution of reentrant arrhythmia dynamics early after initiation. The optical images showed that these changes were associated with marked destabilization of rotors. The NF-induced modification of spiral-wave dynamics may be the result of repolarization delay. All of the action potentials around the center of the reentrant circuit in the absence of NF (control) were elicited successively with no isoelectric segments between excitations, suggesting no substantial excitable gap. The prolongation of APD in such reentrant circuits should lead to a tremendous increase in interactions between the wavefront and wave tail. The functional block line formed by the refractory wake of the wave moving in the opposite direction has to be prolonged to maintain the rotation. The wavefront would encounter its own tail more frequently and move toward more excitable tissue, giving rise to complex meandering of the circuit by offspring of the wavefronts. This sequence of events has been visualized clearly by constructing wavefront–tail and phase maps.

**Figure 6.** Spiral-wave reentry termination by mutual annihilation and exit from the ventricles. A, Spontaneous termination of VT in the absence of nifekalant (control) as a result of mutual annihilation of counter-rotating spiral-waves (#). Left, Four snapshots of phase maps of the final beat of a VT episode. PSs are indicated by circles (black for clockwise rotation, white for counterclockwise rotation). Middle, Trajectories of the 2 PSs plotted on space (x, y) and time axes (red for clockwise rotation, blue for counterclockwise rotation). Right, Optical action potential signals recorded from 6 sites (a through f) indicated at the left. B, Spontaneous termination of VT in the presence of nifekalant by exit of a rotor from the ventricles. Left, Four snapshots of phase maps of the last beat. Cross-hatched bar at the top of each frame indicates the atrioventricular groove. Redline indicates trajectory of a PS. Middle, Trajectory of a PS plotted on space (x, y) and time axes (the blue wall on the right indicates the atrioventricular groove). Right, Optical action potential signals recorded from five sites (a through e) indicated at the left.
Spiral-Wave Reentry Termination

Information on the effect of IKr block on the dynamics of spiral-wave reentry in the ventricular myocardium is still limited. An analysis of computer model of isotropic 2D cardiac tissue showed discrepancies among investigators in the role of the delayed rectifier K⁺ current (IKr) in the regulation of spiral-wave dynamics. In their model, Beaumont and Jalife21 showed that APD is significantly abbreviated (too short for IK activation) near the center of the rotation. Thus, IKr block prolongs APD only in the periphery and not close to the center. This leads to frequent wavefront–tail interaction in the spiral arm without affecting the rotation period. Jalife and colleagues21,24 suggested that the inward rectifier K⁺ current (IK1) may play a much more important role than IKr (IKr and IKs) in regulation of spiral core dynamics. In their simulation using the phase 1 Luo–Rudy ventricular action potential model, on the other hand, Qu et al25 have shown that APD close to the spiral core is longer than in the periphery, and a substantial amount of IK is preserved at the rotation center. They demonstrated that reduction of IK conductance promotes meandering of the spiral core and that quasiperiodic meandering is converted to chaotic meandering that culminates in the breakup of rotors.25

Our observations in the rabbit heart suggest that IKr plays an essential role in repolarization of the action potential not only in the arm but close to the core of spiral-wave reentry. In the absence of NF (control), most spontaneous terminations of spiral-type excitations were the result of mutual annihilation of a pair of rotors with opposite chiralities. NF facilitated the spontaneous termination by 2 different mechanisms: extinction of rotor(s) after collision with the anatomic boundary and trapping of the spiral tip in a region entirely surrounded by refractory tissue. The former mechanism is attributable to considerable meandering of the rotation center, whereas the latter is attributable to APD prolongation of the preceding excitation. The latter mode of termination is similar to that reported by Beaumont and Jalife21 in their 2D cardiac tissue model when sodium current inactivation was slowed in combination with APD prolongation or when the outward component of IK1 was reduced. In a recent theoretical study by Qu and Weiss,26 blockade of time-dependent K⁺ channel was shown to increase dynamic instability of rotors and to facilitate their self-termination. The present results validate their prediction.

Limitations

In this study using a 2D subepicardial layer of rabbit ventricular myocardium, NF destabilized spiral-wave reentry

![Figure 7. Spiral-wave reentry termination by trapping the wavefront. A, Isochrone and APD90 maps of the final 3 beats of VT before spontaneous termination in the presence of nifekalant (NF). Isochrones are at 5.33-ms intervals (green lines for earlier wavefronts, blue lines for later wavefronts). The latest activation front is presented by a dotted red line, and the line of functional block is shown in yellow. APD90 in the recording area is shown as a color gradients, ranging from red (shortest) to blue (longest). B, Optical action potential signals recorded at 2 sites (sites a and b in A). Numerals at the bottom of each action potential indicate the APD90 (ms). C, Snapshots of phase maps in the final beat. PS with clockwise rotation is indicated by black circle. D, Trajectory of the PS plotted on space (x, y) and time axes.](http://circres.ahajournals.org/Content/9/2/F5.jpeg)
in the heart through a modulation of repolarization, leading to its early termination or breakup. Extending these results to larger 3D human hearts is not straightforward. If there is sufficient tissue mass, the chance of spontaneous termination of rotors by wavefront collision or trapping would be reduced, whereas the enhancement of rotor meander and wave instability may promote breakup in favor of a transition from VT to VF. A greater structural discontinuities and functional heterogeneities in diseased hearts would also alter the spatial requirements of spontaneous termination. Thus, NF can be not only antiarrhythmic but also proarrhythmic. We used BDM as an excitation–contraction uncoupler, which is known to inhibit many ionic currents and to reduce the APD restitution slope.\(^\text{27}\) However, this does not seem to invalidate the present results, because the characteristic modification of the spiral-wave dynamics by NF was preserved in the absence of BDM. There are considerable species differences in the relative contribution of \(\text{IKr}\) to the repolarization of action potentials in ventricular myocytes. These limitations should be taken into account when applying the observations in this study to the clinical use of NF.

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