Mechanisms of destabilization and early termination of spiral wave reentry in the ventricle by a class III antiarrhythmic agent, nifekalant

Masatoshi Yamazaki,¹,² Haruo Honjo,¹,³ Harumichi Nakagawa,¹ Yuko S. Ishiguro,¹ Yusuke Okuno,¹ Mari Amino,²Ichiro Sakuma,³ Kaichiro Kamiya,¹ and Itsuo Kodama¹

¹Research Institute of Environmental Medicine, Nagoya University, Nagoya; ²Department of Cardiology, Tokai University School of Medicine, Isehara; and ³Graduate School of Technology, The University of Tokyo, Tokyo, Japan

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Yamazaki M, Honjo H, Nakagawa H, Ishiguro YS, Okuno Y, Amino M, Sakuma I, Kamiya K, Kodama I. Mechanisms of destabilization and early termination of spiral wave reentry in the ventricle by a class III antiarrhythmic agent, nifekalant. Am J Physiol Heart Circ Physiol 292: H539–H548, 2007. First published August 25, 2006; doi:10.1152/ajpheart.00640.2006.—Nifekalant (NF) is a novel class III antiarrhythmic agent that is effective in preventing life-threatening ventricular tachycardia/fibrillation (VT/VF). We investigated mechanisms of destabilization and early termination of spiral-type reentrant VT by NF in a two-dimensional subepicardial myocardial layer of Langendorff-perfused rabbit hearts (n = 21) using a high-resolution optical action potential mapping system. During basic stimulation, NF (0.1 μM) caused uniform prolongation of action potential duration (APD) without affecting conduction velocity and an increase of APD restitution slope. VTs induced by direct current stimulation in the presence of NF were of shorter duration (VTs increased by 10.2 ± 0.3 ms after NF vs. 19 ± 9 ms control). During VTs in control (with visible rotors), the wave front chased its own tail with a certain distance (repolarized zone), and they seldom met each other. The average number of phase singularity (PS) points was 1.31 ± 0.14 per 665 ms (n = 7). In the presence of NF, the wave front frequently encountered its own tail, causing a transient breakup of the spiral wave or sudden movement of the rotation center (spatial jump of PS). The average number of PS was increased to 1.63 ± 0.22 per 665 ms (n = 7, P < 0.05) after NF. The mode of spontaneous termination of rotors in control was in most cases (9/10, 90%) the result of mutual annihilation of counterrotating wave fronts. With NF, rotors frequently terminated by wave front collision with the atrioventricular groove (12/19, 63.2%) or by trapping the spiral tip in a refractory zone (7/19, 36.8%). Destabilization and early termination of spiral wave reentry induced by NF are the result of a limited proportion of excitable tissue after modulation of repolarization.

potassium channel blocker; optical mapping; ventricular tachycardia

VENTRICULAR TACHYCARDIA/FIBRILLATION (VT/VF) is the major cause of sudden cardiac death. It is generally believed that regardless of the initiating event, spiral- or vortex-type reentrant activity rotating around a functional obstacle ( rotor) is the major organization center of VT/VF (3, 22, 25–27). Pharmacological regulation of such rotors is therefore the central task to be achieved for efficient prevention of sudden arrhythmic death (8, 22, 25, 26). 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 (I_{Kd}) (12, 16, 17), and at higher concentrations, NF has an inhibitory effect on other voltage- and ligand-gated K⁺ currents (12, 16). APD prolongation by pharmacological blockade of I_{Kd} renders a certain proarrhythmic propensity known as drug-induced QT prolongation and torsades de pointes, and this potential risk limits the use of class III antiarrhythmic drugs (20). In experimental animals, however, NF has been shown to prevent VT/VF after acute myocardial infarction without compromising hemodynamics (9) and to improve electrical defibrillation efficacy (14). 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 direct current (DC) shocks (15, 16).

In a recent optical mapping study to examine the combined effects of NF and lidocaine in a two-dimensional (2D) subepicardial layer of rabbit ventricular myocardium, we have demonstrated that NF (0.5 μM) alone prolongs VT cycle length and causes its early termination in association with destabilization of the spiral wave dynamics (prolongation of functional block line, frequent local conduction block, and extensive meandering) (1). Preliminary experiments with NF at a lower concentration (0.1 μM) also showed analogous modification of spiral wave reentry (10). On the basis of these observations, we speculated that the NF-induced destabilization of spiral wave reentry may be the result of repolarization delay leading to more frequent wave front-tail interactions, but the issue remains to be substantiated. The mechanism of early VT termination was unclear. The present study was designed to clarify the points in similar 2D rabbit ventricular myocardial tissue preparations. Newly developed software to visualize the wave front and tail of optical action potential signals was employed to analyze their interaction. A phase-mapping method was used to quantify the destabilization and to elucidate the mode of termination of rotors. The results revealed that the wave front during VTs in the presence of NF does frequently encounter its own tail, giving rise to transient breakup of spiral wave or sudden movement of the rotation center, and that early VT termination with NF is the result of either wave front collision with the atrioventricular groove or trapping of the spiral tip in a refractory zone.

METHODS

Experimental preparations. The protocol was approved by the Institutional Animal Care and Use Committee at Nagoya University.

* M. Yamazaki and H. Honjo contributed equally to this work.

Address for reprint requests and other correspondence: I. Kodama, Dept. of Cardiovascular Research, Research Institute of Environmental Medicine, Nagoya Univ., Chikusa-ku, Nagoya 464-8601, Japan (e-mail: ikodama@riem.nagoya-u.ac.jp).

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Experiments were performed in vitro on hearts obtained from Japanese White rabbits of both sexes weighing 1.7–2.0 kg. The experimental procedure was described previously in detail (1). In brief, rabbits were anesthetized with pentobarbital sodium (10–15 mg/kg), and the hearts were rapidly removed. The isolated hearts were continuously perfused on a Langendorff apparatus with modified Krebs-Ringer solution equilibrated with 95% O2–5% CO2 to maintain pH 7.4 (37°C). Complete atrioventricular block was produced by ligation of the His bundle. We created a 2D epicardial layer of ventricular myocardium by performing a cryoprocedure (1, 10). This model is similar to that reported by Schalij et al. (23) and has an advantage over use of the intact three-dimensional heart to visualize the spiral wave reentry on the epicardial surface. At the end of the experiment, the heart was stained with 2,3,5-triphenyltetrazolium chloride (TTC) and sectioned parallel to the atrioventricular groove, from base to apex, at 2-mm intervals. The surviving myocardium, which was stained deeply with TTC (reservation of dehydrogenase activity), was 1.0 ± 0.2 mm thick (10 hearts).

High-resolution optical mapping. The optical mapping system used in this study was described previously (1). After endocardial freezing, the hearts were stained with a voltage-sensitive dye, di-4-ANEPPS. To minimize motion artifacts, we added 15 mM 2,3-butanedione monoxime (BDM) unless otherwise specified. Bipolar electrograms were recorded through widely spaced electrodes, one at the apex of the left ventricle and the other at the lateral wall of the right ventricle, for 750 frames/s. The images covered the anterolateral surface of the left ventricle and part of the anterior surface of the right ventricle. Each acquisition lasted 10.9 s.

To reveal the signal, we subtracted the background fluorescence from each frame and applied low-pass spatial filtering. The fluorescence signals were inverted and then spatially averaged to reduce noise. Spatial resolution after the low-pass filtering was 0.36–0.48 mm. Isochrome maps were generated from the filtered image. Action potential configuration was analyzed after the application of a five-point median filter to the spatially averaged data (13), and then the data were normalized to the range of the maximum and the minimum values in the respective 1,000-frame sample. A time point at 10% depolarization in the upstroke phase and a time point at 90% repolarization in its repolarization phase were identified for each action potential signal, and their interval was measured as APD at 90% repolarization (APD90). The distribution of APD90 values in the recording area was displayed as a color gradient map with 1.33-ms steps, ranging from red (shortest) to blue (longest).

Wave front-tail dynamics during VT were visualized by connecting the 10% depolarization points in the action potential upstrokes as the wave front (red) in the recording area and by connecting the 90% repolarization points as the wave tail (green). We quantified the pattern of wave propagation during VT using the phase-mapping method described by Gray et al. (5). The fluorescence of each pixel at time t, F(t), was plotted against the fluorescence of the same pixel offset by a time interval τ = 25 frames. A cyclic return map of F(t) vs. F(t − τ) was constructed, allowing a new parameter, phase θ(t), to be defined as the angle of the coordinate [F(t), F(t − τ)] around the mean fluorescence for that given pixel, with values between −π and π. After the transformation, a new phase θ(t) movie was produced that included all pixels, in which different phases of the action potential were represented by a continuous color gradient extending from red to purple. Phase singularity (PS) was defined as the point at which all phases converged.

Experimental protocols. Conduction velocity and APD90 were measured during constant (S1) stimulation at the center of the left ventricular free wall at basic cycle lengths (BCLs) of 180–800 ms. A monopolar electrode made of platinum wire was used for stimulation. The longitudinal (L) and transverse directions (TD) of propagation were determined from the activation maps elicited by S1 stimulation (1). Conduction velocity was measured in a central 18 × 18-mm square around the stimulation site, since measurement in the outer periphery would have been hampered by the sharp curvature of the ventricular surface. Conduction velocities in LD and TD were calculated from the slope of a linear least-squares fit of the activation time plotted against the distance. Data from an area very close to the stimulation site (<2 mm) were excluded to minimize the virtual electrode polarization effects.

We used the dynamic pacing method (11) to characterize the APD restitution properties. The center of the left ventricular free wall was initially paced at a BCL of 400 ms, and the BCL was progressively shortened in steps of 5–20 ms. A minimum of 30 stimuli were delivered at a given BCL. APD restitution at a site 5 mm from the stimulation site in TD was analyzed. The APD90 of the final paced action potential was measured at a BCL with no APD alternans. When APD alternans occurred at a shorter BCL, the pacing was interrupted twice to measure the APD90 of both long and short action potential. The BCL was shortened until either a 2:1 block or higher order periodicity occurred. The APD restitution curve was constructed by plotting APD90 against the preceding diastolic interval, and the curve was fitted to a single-exponential function (Origin 7.0; Microcal Software, Northampton, MA).

VTs resulting from spiral-type excitation were induced by modified cross-field stimulation. Eighteen S1 stimuli at a BCL of 400 ms were applied to the apex through a pair of contiguous bipolar electrodes. A 10-ms monophasic rectangular pulse of constant voltage (20 V) was generated by a DC power unit and was delivered from the unit through a 6,700-μF capacitor connected to a power MOS-FET switch. A pair of Ag-AgCl paddle electrodes (7 mm in diameter) were placed on the lateral surface of the left and right ventricles for the DC stimulation (S2) to induce electrical field roughly perpendicular to the S1 excitation from the apex to the base (modified cross-field stimulation). The S1-S2 coupling interval was varied at 10-ms steps to apply S2 during the vulnerable window of the final S1 excitation.

Data were obtained before (baseline) and 20–30 min after application of 0.1 μM NF (Nihon Schering, Osaka, Japan). All the experiments were completed within 120 min of perfusion, during which the conduction velocity, APD90, and APD restitution were unchanged (see Supplemental Table 1; the online version of this article contains supplemental data). We confirmed in pilot experiments of four rabbit hearts that the effects of NF (0.1 μM) on the action potential configuration reached a steady state at ~30 min (see Supplemental Table 2). At the end of experiment, the tissues were fixed in 15% formalin and sectioned parallel to the epicardium at a 8-μm interval. The sections were stained with hematoxylin and eosin to examine the fiber orientation in the mapped area.

Statistical analysis. Group data are expressed as means ± SD. Statistical comparisons were performed using two-way ANOVA with Tukey’s test, 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. Conduction properties and action potential configurations were examined in seven hearts during constant stimulation from a center of the left ventricular free wall at a wide range of BCLs (180–800 ms). The isochrones of activation front exhibited a smooth, symmetric, elliptical pattern; the long axis corresponded to the fiber orientation of subepicardial cardiac muscle. In the central 18 × 18-mm square, there was a linear correlation between
activation times and distances in both LD and TD. Conduction velocity in the LD and TD under control conditions was 55.2 ± 4.3 and 23.1 ± 2.1 cm/s, respectively, at a BCL of 800 ms. The values decreased slightly at shorter BCLs and reached 42.4 ± 3.9 and 16.6 ± 0.8 cm/s, respectively, at a BCL of 180 ms. The anisotropic ratio of the velocity was 2.4 to 2.6. NF at 0.1 μM and caused no significant changes in these parameters.

Figure 1A shows representative changes in APD in response to NF application. Action potential signals obtained from 16 sites covering an 18 × 18-mm square were superimposed. NF (0.1 μM) caused uniform prolongation of APD at all recording sites. Figure 1B summarizes the changes in APD<sub>90</sub> (average of the values at the 16 sites). NF caused significant prolongation of APD<sub>90</sub>, and the prolongation was greater at longer BCLs. The dispersion of the APD<sub>90</sub> values among the 16 recording sites was virtually unaffected (Fig. 1C).

Figure 1, D and E, shows representative data for the APD restitution. The plots of APD<sub>90</sub> against the diastolic intervals before and after NF (0.1 μM) both fitted single-exponential function well (Fig. 1E). 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 five 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).

VTs induced by modified cross-field stimulation. VTs were induced in 15 hearts by DC stimulation before (control) and after NF (0.1 μM) application. In controls, 74 of 93 VTs (79.6%) terminated spontaneously within 30 s (nonsustained), whereas the other 19 (20.4%) persisted >30 s (sustained). Forty-six (49.5%) of the 74 nonsustained VTs terminated within 5 s. Of the 54 VTs in the presence of NF, 52 VTs (96.2%) were nonsustained and the 2 other VTs (3.7%) were sustained. Forty-four (81.5%) of the 52 nonsustained VTs terminated within 5 s. Thus VTs induced in the presence of NF (0.1 μM) terminated earlier than in the control conditions. VT cycle length (VTCL) after NF was significantly longer than under the control conditions (188 ± 31 vs. 154 ± 16 ms, P < 0.05).
Optical images of excitation during VT were analyzed in nine hearts exhibiting visible rotors in the observation area under the control conditions as well as after NF. Figure 2 shows a representative experiment. The record under the control condition (Fig. 2A) was obtained 2 s after VT initiation (see Supplemental Movie 1). Clockwise rotation of wave fronts circulating around a line of functional block (~7 mm) with a VTCL of 141 ms can be seen in isochrone maps. The circuit was more or less stable for >10 s and exhibited minimal meandering. A bipolar electrogram obtained during the VT episode showed monomorphic ventricular excitations. Characteristic features of the action potential configuration around the circuit, which were reported previously (1, 10), are recognized; those include slower upstroke at sites close to the pivot points (sites b and d), faster upstroke at sites after passing through the pivot points (sites a and c), clear double potential at the center of functional block line (site e), and no isoelectric segments between action potential signals. The block lines (4–9 mm) run either parallel (~70%) or across (~30%) the fiber orientation. There was no obvious tissue damage or macroscopic structural discontinuity to anchor the reentrant pathway in a fixed position.

Figure 2B shows the activation pattern during a short VT (lasting for 3 s) induced after application of NF (0.1 μM) (see Supplemental Movie 2). Rotors circulating around functional block lines were observed in isochrone maps during the three consecutive cycles, but their circuits changed dramatically in a beat-to-beat manner, with VTCLs varying from 176 to 195 ms. In beat 1, a wave front coming down from the base faced a long oblique functional block line (site b), giving rise to its extension toward the right margin. The wave, after turning around the right margin, was divided into dual circuits, one toward the base and another toward the apex and the left margin. The upper circuit showed a local conduction block (site e), causing a further extension of the upper block line to maintain the clockwise rotation. In beat 2, the clockwise rotation of different dual circuits was preserved; the upper circuit was around a long functional block line in a direction (along the fiber orientation) and configuration similar to those of beat 1, whereas the lower circuit was around an L-shaped...
functional block line (one half along and one half across the fiber orientation). In beat 3, the upper functional block line moved toward the posterior surface (visible line was shortened). On the other hand, the lower functional block line was largely prolonged, traversing the whole anterior surface. Isochrones in the observation area for beat 3 showed an almost single large clockwise rotation. The total length of visible functional block lines for the three beats was 23.7–35.2 mm. The rotor terminated spontaneously five cycles later. Bipolar electrograms obtained during the VT episode showed polymorphic torsades de pointes-like ventricular excitations. The action potential signals from the circuits showed a marked beat-to-beat variation that reflects complex meandering of rotors and frequent conduction block.

Qualitatively similar results were obtained in all nine hearts. Thus the characteristic effects of NF on the spiral wave dynamics (prolongation of functional block line, an increase in meandering of circuits, and earlier termination of rotation) (1) have been visualized clearly in experiments at a lower drug concentration (0.1 μM).

Wave front-tail interaction and phase singularity. The mechanisms of NF-induced modification of spiral wave reentry were investigated in terms of the wave front-tail interaction and the PS dynamics. Seven VT episodes with rotors visible in the observation area were analyzed for each before (control) and after application of NF. During VTs under the control conditions, the wave front chased its own tail with a certain distance between them (repolarized zone), and the wave fronts seldom met each other (Fig. 3A, top). The number of PS points in the phase maps was normally (>90%) 1 (a single rotor; Fig. 3A, bottom) and, rarely, increased to 2 for a short period (Fig. 3D). In the presence of NF (0.1 μM), the wave front frequently encountered its own tail, causing transient breakup of the spiral wave (Fig. 3B, top) or sudden movement of the organizational center of rotation to another site (Fig. 3C, top). In the phase maps, the former was recognized as an increase in PS from 1 to 3 (Fig. 3B, bottom), whereas the latter was recognized as a sudden jump in PS site (Fig. 3C, bottom). Figure 3D shows representative changes in PS number per 500 frames (665 ms) after application of NF. Pooled data are summarized in Fig. 3E.

Fig. 3. Wave break formation by wave front-tail interaction. A: wave front (red) chasing a wave tail (green) during VT in the control condition. The top and bottom images are snapshots of wave front-tail and corresponding phase maps, respectively. The wave front does not encounter the tail (no breakup). B: interaction of a wave front with a wave tail during VT after application of 0.1 μM NF, causing the transient breakup of a rotor. C: interaction of a wave front with a wave tail during VT after NF, causing a sudden movement of the organizational center to another site. Phase singularity points (PSs) are indicated by circles (black for clockwise rotation, white for counterclockwise rotation). D: number of PSs over 500 frames (665 ms) during VT before (top) and after NF (bottom) (from the same heart as in A–C). E: average number of PSs/500 frames (665 ms) before (control) and after NF (means ± SD, n = 7). *P < 0.05 vs. control.
the average number of PS points 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 counterrotating spiral waves. Figure 4A shows representative experiments (see Supplemental Movie 3). Excitation patterns for the final beat of a VT episode are shown in four sequential phase maps (left). A pair of PS points with opposite chiralities constructing a figure eight reentry circuit was present in the lower region of the left ventricle (1,850 ms). The distance between the two PS points initially increased (1,850 to 1,935 ms) and then decreased (1,935 to 1,951 ms), culminating in mutual annihilation (1,961 ms). The trajectories of the 2 PS points plotted on space (x, y) and time axes are shown in the middle (red, clockwise; blue, counterclockwise). Action potential signals recorded from six sites in the figure 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 (atrioventricular groove).

In the presence of NF, 12/19 VTs (63.2%) terminated by rotor extinction after considerable meandering toward the anatomic boundary. Figure 4B shows an example (see Supplemental Movie 4). In the four sequential phase maps (left), a clockwise rotating PS initially moved a long distance from the upper right region to the right margin, then back toward the upper central region (1,282 to 1,382 ms), and was finally pushed out of the atrioventricular groove (1,463 to 1,490 ms). The trajectory of the PS plotted on space and time axes is shown in the middle (the blue wall at right indicates the atrioventricular groove), and action potential signals recorded from five sites in the meandering pathway are shown at right.

In the remaining seven VTs (36.8%) in the presence of NF, the rotors terminated by trapping the spiral tip in a region at the atrioventricular groove.

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**Fig. 4.** Spiral wave reentry terminated by mutual annihilation and exit from the ventricles. **A**: spontaneous termination of VT in the absence of NF (control) as a result of mutual annihilation of counterrotating spiral waves (#). **Left**, 4 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 from 6 sites (a–f) indicated at left. **B**: spontaneous termination of VT in the presence of NF by exit of a rotor from the ventricles. **Left**, 4 snapshots of phase maps of the last beat; crosshatched bar at top of each frame indicates the atrioventricular groove, and red line indicates the trajectory of a PS. **Middle**, trajectory of a PS plotted on space (x, y) and time axes (blue wall at right indicates the atrioventricular groove). **Right**, optical action potential signals recorded from 5 sites (a–e) indicated at left.
entirely surrounded by refractory tissue. Figure 5 shows a representative experiment. Isochrone and APD$_{90}$ maps of three beats before termination are shown in Fig. 5A. 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 wave front from the left upper region was blocked in the central region. Because this wave front (red dotted line) was entirely surrounded by a refractory zone, no offspring wavelets emerged, and the VT terminated. Action potential signal tracings from two sites (asterisks) revealed APD alternans during the VT cycles (Fig. 5B), with the longest APD$_{90}$ of beat 3 (site a) preventing further rotation of the wave front. The APD 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 seven VTs of this group. Figure 5C shows phase maps of the final beat (see Supplemental Movie 5). The PS of clockwise rotation shifted from the left upper region to the center and then disappeared. The trajectory of the PS plotted on space and time axes is shown in Fig. 5D.

VTs induced in the absence of BDM. In three hearts, VTs were induced in the absence of BDM, and the effects of NF (0.1 µM) on the spiral wave dynamics were analyzed using phase maps. The results in each of the three hearts were essentially similar to those obtained in the presence of BDM. In controls, 27 of 32 VTs (84.4%) terminated spontaneously within 30 s (nonsustained), whereas the other 5 VTs (15.6%) persisted >30 s (sustained). Of these VTs, 26 (81.3%) terminated within 5 s. All 12 VTs (100%) in the presence of NF were nonsustained and terminated within 5 s.

A representative experiment is shown in Fig. 6. In the control conditions (Fig. 6A), a PS of counterclockwise rotation was present in the middle of the anterior surface of the left ventricle. A trajectory of the PS revealed that the rotor was more or less stable and exhibited moderate meandering (1,718–2,041 ms). After application of NF (Fig. 6B), a PS of clockwise rotation moved a long distance with a complex trajectory; from the right margin to the upper central region.

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**Fig. 5.** Spiral wave reentry termination by trapping the wave front. A: isochrone and APD$_{90}$ maps of the final 3 beats of VT before spontaneous termination in the presence of NF. Isochrones are at 5.33-ms intervals (green lines for earlier wave fronts, blue lines for later wave fronts). The latest activation front is presented by a dotted red line, and the line of functional block is shown in yellow. APD$_{90}$ in the recording area is represented by color gradients, ranging from red (shortest) to blue (longest). B: optical action potential signals recorded at 2 sites (sites a and b in A). Numbers at the bottom of each action potential indicate the APD$_{90}$ (ms). C: snapshots of phase maps in the final beat. PS with clockwise rotation is indicated by a black circle. D: trajectory of the PS plotted on space (x, y) and time axes.
(895–942 ms), going back to the right margin, followed by a traverse in opposite direction toward the posterior surface. The PS reappeared on the anterior surface (left upper region), turned back toward the left upper corner (anatomical boundary), and terminated (1,127–1,250 ms). Action potential signals showed nearly uniform configuration in control (Fig. 6A) compared with marked beat-to-beat variation after NF (Fig. 6B).

**DISCUSSION**

The results of this study in a 2D ventricular myocardium with normal anisotropy have revealed that NF-induced delay and dynamic instability of ventricular repolarization causes frequent collision of spiral wave fronts with their own tails or anatomical boundaries, giving rise to breakup, sudden movement of PS, or extinction of rotors.

**Action potential and conduction properties.** In this study, we first examined the effects of 0.1 μM NF on the conduction velocity and APD under constant stimulation at a wider range of BCLs (180–800 ms) than in our previous study at 0.5 μM (BCLs 250 and 400 ms) (1). NF (0.1 μM) caused spatially uniform APD prolongation without affecting conduction velocities. The APD prolongation was significant even at a BCL of 180 ms, but it was greater at longer BCLs. The spatial dispersion of APD measured at 16 sites to cover an 18 × 18-mm square was unaffected by NF. NF increased the slope of the APD restitution curve, and the change was associated with an increase in APD alternans. All of these NF-induced alterations in the steady-state and dynamic electrophysiological properties of ventricular muscle can be ascribed to $I_{Kr}$ blockade. In action potential clamp experiments, Hua and Gilmour (7) recently demonstrated that $I_{Kr}$ contribute 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.** In our 2D ventricular muscle tissue preparations of rabbit heart, spiral wave reentry rotating around functional block lines (single or double loops) can be induced by modified cross-field stimulation in the observation area in more than half (53–61%) of whole VT episodes under the control conditions (1, 10). The VTs induced are polymorphic during the initial several beats (1, 10) but become almost monomorphic during the subsequent beats. 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 (0.1 μM) are, consistent with our previous report with NF at 0.5 μM (1), characterized by longer cycle length, polymorphic configuration, and earlier termination than those observed in the controls. The optical images showed that these changes were associated with marked destabilization of rotors, which circulated around much longer and variable functional block lines formed by refractory wake of preceding excitations. We analyzed movies of wave front and wave tail during the rotations and found that a wave front in the presence of NF (0.1 μM) frequently encounters its own tail, resulting in breakup or sudden jump of organizational center of the rotation. The marked destabilization of rotors in the presence of NF cannot be ascribed to a APD restitution slope >1, since the maximum slope after NF, although increased, remained 0.7 on average. Other factors such as short-term cardiac memory, electronic currents, and unstable intracellular Ca2+ cycling also can affect wave stability and may have played a role in enhancing meander of the circuit. The enhanced meandering may have further facilitated the wave front-tail interactions through creation of Doppler-shifted short cycle length in front of moving phase singularities (4, 6).

**Spiral wave reentry termination.** Information on the effect of I_{Kr} 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 (I_{K}) in the regulation of spiral wave dynamics. In their model, Beaumont and Jalife (2) showed that APD is significantly shortened (too short for I_{K} activation) near the center of the rotation. Thus I_{K} block prolongs APD only in the periphery and not close to the center. This leads to frequent wave front-tail interaction in the spiral arm without affecting the rotation period. Jalife and colleagues (2, 21) suggested that the inward rectifier K+ current (I_{K1}) may play a much more important role than I_{K} [I_{K} and I_{Ks} (slow component)] 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 al. (18) have shown that APD close to the spiral core is longer than in the periphery and that a substantial amount of I_{K} is preserved at the rotation center. They demonstrated that reduction of I_{K} conductance promotes meandering of the spiral core and that quasiperiodic meandering is converted to chaotic meandering that culminates in the breakup of rotors (18).

Our observations in the rabbit heart suggest that I_{K} 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 two 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 mode is attributable to considerable meandering of the rotation center, whereas the latter is attributable to APD prolongation of the preceding excitation in association with increased APD alternans near the spiral tip. The latter mode of termination is similar to that reported by Beaumont and Jalife (2) in their 2D cardiac tissue model when sodium current inactivation was slowed in combination with APD prolongation or when the outward component of I_{K1} was reduced. In a recent theoretical study by Qu and Weiss (19), 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.

Most of the modification of spiral wave dynamics induced by NF (0.1 μM) may be shared by other I_{Kr} blocking drugs, since much higher concentrations (5–100 μM) are required for NF to affect other voltage- and ligand-gated K+ currents (17). However, further experimental studies are required to elucidate the point.

**Limitations.** In this study using a 2D subepicardial layer of rabbit ventricular myocardium, NF-induced delay and instability of ventricular repolarization were shown to cause frequent collision of spiral wave fronts with their own tails or anatomical boundaries, giving rise to breakup or early extinction of rotors. Extending these results to 3D hearts, especially in larger animals including humans, is not straightforward. If there is sufficient tissue mass, the chance of spontaneous termination of rotors by wave front collision or trapping would be reduced, whereas the enhancement or rotor meander and wave instability may promote breakup in favor of transition from VT to VF. The greater structural discontinuities and functional heterogeneities in diseased hearts would also alter the spatial requirements of spontaneous rotor termination. Thus NF can be not only antiarrhythmic but also proarrhythmic. There are certain clinical reports (in Japanese) showing excessive QT prolongation and torsades de pointes induced by NF (0.1–0.3 mg/kg iv). Plasma concentrations at such therapeutic doses are comparable to the NF concentration (0.1 μM) employed in the present study (17). The proarrhythmia via rotor breakup might be dangerous, since it would promote VF generation and perpetuation. We used BDM as an excitation-contraction uncoupler that is known to alter ionic currents and to reduce the APD restitution slope (13). 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. We cannot completely neglect the potential photodynamic toxicity of the voltage-sensitive dye (di-4-ANEPPS), but this may not have significant effects on the electrophysiological properties of the preparations in our experimental conditions, because there were no time-dependent changes of conduction velocity and action potential configuration up to 120 min of control perfusion (see Supplemental Table 1). There are considerable species differences in the relative contribution of I_{Kr} to the repolarization of action potential in ventricular myocytes. Despite these limitations, the present results may provide a new perspective for future development of drugs to prevent sudden arrhythmic death.

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**References**


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