Combined Effects of Nifekalant and Lidocaine on the Spiral-Type Re-Entry in a Perfused 2-Dimensional Layer of Rabbit Ventricular Myocardium

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Background  Spiral re-entry plays the principal role in the genesis of ventricular tachycardia and ventricular fibrillation (VT/VF). The specific I_{Kr} blocker, nifekakant (NIF) has, often in combination with lidocaine (LID), recently been used in Japan to prevent recurrent VT/VF, but the combined effects of these drugs on spiral re-entry had never been investigated.

Methods and Results  A ventricular epicardial sheet was obtained from 13 Langendorff-perfused rabbit hearts by means of a cryoprocedure, and epicardial excitations were analyzed with a high-resolution optical mapping system. Nifekakant (0.5 μmol/L) caused significant prolongation of action potential duration (APD) and LID (3 μmol/L) attenuated the APD prolongation without affecting the conduction velocity. VT were induced in 6 hearts by cross-field stimulation, and single- or double-loop spirals circulating around variable functional block lines were visualized during the VT. Nifekakant reduced VT cycle length and caused early termination in association with destabilization of the spiral dynamics (prolongation of functional block line, frequent local conduction block, and extensive meandering). These modifications of spiral-type re-entrant VT by NIF were prevented by addition of LID.

Conclusions  The effects of NIF on the spiral excitations are reversed by LID. This interaction should be taken into account when these drugs are used in combination to treat VT/VF. (Circ J 2005; 69: 576–584)

Key Words:  Lidocaine; Nifekalant; Optical mapping; Spiral re-entry; Ventricular tachycardia

Spiral-type excitation is the principal mechanism of functional re-entry for the genesis of life-threatening ventricular tachycardia and ventricular fibrillation (VT/VF). Nifekakant (NIF) hydrochloride 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 potassium current (I_{Kr}). The 2000 American Heart Association Guidelines for cardiopulmonary resuscitation (CPR) recommends intravenous amiodarone to prevent recurrent VT/VF, but nifekakant and LID have not yet been approved for CPR in Japan and nifekakant (NIF) is used, often in combination with lidocaine (LID), as an alternative. Nifekakant and LID are also used sequentially to treat recurrent VT/VF resistant to DC shocks; however, little information is available on the electropharmacological basis for the combined use of these drugs. The present study was designed to investigate the effects of NIF and NIF+LID on the spiral-type excitation in a perfused 2-dimensional (D) layer of rabbit ventricle by using our custom-made high-resolution optical mapping system.

Methods

Experimental Model

Japanese White rabbits of both sexes weighing 1.7–2.0 kg (n=13) were anesthetized with thiamylal sodium (10–15 mg/kg), and after opening the thorax, the heart was rapidly removed. Complete atroventricular block was produced by ligating the His bundle with fine silk thread. The aorta was then cannulated, and the heart was connected to a Langendorff perfusion apparatus. The coronary arteries were perfused with modified Krebs Ringer solution at a constant flow (35–45 ml/min). The solution was equilibrated with 95% O_2 and 5% CO_2 to maintain its pH at 7.4, and its temperature was maintained at 35°C.

Because of its 3-D structure with variable filament configuration, vortex-type excitations appear only transiently and incidentally in the intact heart, thereby hampering detailed analysis of spiral dynamics by optical mapping. To avoid this problem, we created a 2-D preparation by the method described by Schalij et al. In brief, the Langendorff-perfused rabbit heart was immersed in a tissue bath containing perfusion fluid at 35°C, and a cryoprobe made of copper was inserted into the left ventricular cavity, and the probe was then filled with liquid nitrogen (−192°C) for 5 min. This freezing procedure destroyed the endocardial and intramural layers of the free wall of the left ventricle and the total interventricular septum, leaving only
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a thin left ventricular epicardial layer intact. At the end of the experiment the heart was stained with 2,3,5-,triphenyl-tetrazolium chloride (TTC) and sectioned parallel to the atrioventricular ring from the base to the apex at 2-mm intervals. The thickness of surviving myocardium that stained deeply with TTC (preservation of dehydrogenase activity) was 1.0±0.3 mm (6 hearts).

High-Resolution Optical Mapping

After endocardial freezing the hearts were stained for 10 min with the voltage-sensitive dye di-4-ANEPPS (Molecular Probes), at 2 Жmol/L, added to the perfusate. This initial loading was followed by continuous application of 0.2 Жmol/L di-4-ANEPPS for 120–150 min during the rest of the experiments. To minimize motion artifacts, 15 mmol/L 2,3-butandione monoxime (BDM, Sigma, St Louis, MO, USA) was added to the perfusate.

Fig 1 is a schematic diagram of the optical signal recording system. The vertically hanging heart placed in the Langendorff apparatus was illuminated with blush-green light-emitting diodes (LED). The LED (222 elements in all; NSPE 500S, Nichia) were mounted on a ring-shaped bracket placed in front of the heart. The peak wave length (л) of the excitation light was 505 nm with a half bandwidth (∆л) of ~20 nm. The fluorescence emitted from the epicardial surface of the heart was recorded by a high-speed digital video camera (Fastcam-Ultima 40k, Photron) through a long pass filter (cut-off wavelength of 600 nm). The digital images were stored temporarily in the frame memory (512 megabytes), and transferred to a Windows-based personal computer for off-line analysis in 2-D and scalar display.

Signal Processing

The background fluorescence was subtracted from each frame to reveal the signal. Because of the relatively large background fluorescence, the dynamic range of fluorescence change for each electrical excitation was 12–23 gray levels. The fluorescence signals were inverted and then spatially averaged to reduce noise. The data from 3x3 pixels were averaged at the respective recording site to obtain isochrone maps, whereas the data from 4x4 pixels were averaged to analyze action potential configuration. Spatial resolution after this low-pass filtering was 0.36–0.48 mm.

Isochrone maps were generated from the filtered video imaging data by analyzing the value of each pixel over time. A point in each time series was labeled as part of a wave front, if the first derivative of fluorescence was >30% of the peak value in the respective 100–500 frames sampled. This helped to eliminate most maxima because of noise. The leading edge was identified as the activation wave front from the data points forming a band.

To analyze action potential configuration, a 5-point time median filter was applied to the spatially averaged data, and then the data were normalized to within the range of the maximum and the minimum values in the respective 700–1,000 frames sampled. 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 APD90. The distribution of APD90 values in the recording area was displayed as color gradient maps with 1.33 ms steps ranging from red (the shortest) to blue (the longest).
Experimental Protocols

Conduction velocity (CV) and APD<sub>90</sub> were measured during constant stimulation (S1) from the center of the left ventricular free wall at a cycle length (CL) of 250 ms and 400 ms. A monopolar electrode made of platinum wires was used for stimulation. The pulses applied were 2 ms in duration and had an intensity twice the diastolic threshold. Longitudinal (L) and transverse (T) directions of propagation were determined from the activation maps elicited by S1 stimulation. A line for L propagation was drawn from the pacing site to the outer area of the map, so that it crossed the most widely spaced isochrones (Fig 2A). A second line for T propagation was drawn perpendicular to the first line through the densely spaced isochrones. The CV was measured in a central 15×15 mm square around the stimulation site, as measurement in the outer periphery would be hampered by the sharp curvature of the ventricular surface. The CV in the L and T directions were calculated from the slope of a linear least-square 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.

In experiments to induce VT resulting from spiral-type excitation, 18 S1 stimuli at a CL of 400 ms were applied to the apex through a pair of contiguous bipolar electrodes. A 10 ms DC stimulus (S2, monophasic pulse at 20 V) was then delivered through Ag–AgCl paddle electrodes (5 mm diameter) placed on the lateral surface of the both ventricles. This cross-field S2 stimulation was applied during the vulnerable window of the last S1 excitation. When sustained VTs >30 s were induced, a salvage DC shock was applied through Ag–AgCl paddle electrodes. The CV in long- and transverse directions were measured in a central 15×15 mm square (dotted line) around the stimulation site.

Statistical Analysis

Group data were expressed as mean values ± SE. Statistical comparisons were performed by two-way or one-way analysis of variance (ANOVA with Fisher) when appropriate. Differences were considered significant when the p-value was <0.05.

Results

Conduction Velocity and Action Potential Configuration

Fig 2A shows activation sequences during constant stimulation from the anterior center of the left ventricular free wall (S1–S1 400 ms). At the highest speed the activation front proceeded centrifugally along an oblique line in the left upper and right lower directions, and in directions perpendicular to the line at the slowest speed. As a result, the isochrones of activation showed a smooth and symmetric elliptical pattern. In the rabbit, the long axis of the ellipse has been found to correspond to the fiber orientation of subepicardial cardiac muscle in the anterior left ventricular free wall. In 2 hearts we confirmed that the fiber orientation correlated well with the electrical axis of fast conduction. In the central 15×15 mm square (dotted line), there was a linear relationship between activation times and distances from the stimulation site in either L or T direction. The CV (L and T) and their anisotropic ratio (L/T) were calculated from the slopes.

Application of 0.5 μmol/L NIF alone for 20 min and addition of 3 μmol/L LID for 10 min (NIF + LID) produced no appreciable changes in the uniform anisotropic conduction property (Fig 2B, C). The results obtained from 6 hearts at S1–S1 400 ms and 250 ms are summarized in Table 1. There were no significant differences at either long or short basic CL in L, T, or L/T among the control, NIF, and NIF + LID conditions.

Fig 3 shows representative changes in APD in response to drug application. Left panels are color maps of APD<sub>90</sub> during basic stimulation at the anterior center of the left ventricular free wall. The APD<sub>90</sub> values in the entire mapping area are displayed as color gradients; the shortest APD<sub>90</sub> is colored red, and the longest, blue. Right traces show optical action potential signals recorded from 4 sites...
Table 1  Effects of Nifekalant and Lidocaine on Conduction Velocity During Longitudinal and Transverse Propagation During Constant Stimulation

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>(\Delta L) (cm/s)</th>
<th>(\Delta T) (cm/s)</th>
<th>(\Delta L/\Delta T)</th>
</tr>
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<tbody>
<tr>
<td>S1-S1 400 ms Control</td>
<td>6</td>
<td>55.1±2.7</td>
<td>22.2±1.5</td>
<td>2.53±0.16</td>
</tr>
<tr>
<td>NIF</td>
<td>6</td>
<td>56.9±2.2</td>
<td>22.5±0.8</td>
<td>2.53±0.07</td>
</tr>
<tr>
<td>NIF+LID</td>
<td>6</td>
<td>57.9±3.0</td>
<td>22.4±1.5</td>
<td>2.60±0.09</td>
</tr>
<tr>
<td>LID wash</td>
<td>6</td>
<td>57.1±3.3</td>
<td>23.3±0.8</td>
<td>2.40±0.15</td>
</tr>
<tr>
<td>S1-S1 250 ms Control</td>
<td>6</td>
<td>54.4±3.0</td>
<td>19.7±1.4</td>
<td>2.80±0.20</td>
</tr>
<tr>
<td>NIF</td>
<td>6</td>
<td>55.3±2.0</td>
<td>21.2±1.1</td>
<td>2.64±0.11</td>
</tr>
<tr>
<td>NIF+LID</td>
<td>6</td>
<td>55.8±3.6</td>
<td>21.1±1.6</td>
<td>2.76±0.06</td>
</tr>
<tr>
<td>LID wash</td>
<td>6</td>
<td>55.4±2.7</td>
<td>21.0±1.6</td>
<td>2.68±0.17</td>
</tr>
</tbody>
</table>

Values are means±SE of 6 experiments each. \(\Delta L\) and \(\Delta T\): conduction velocity during longitudinal propagation and transverse propagation, respectively; \(\Delta L/\Delta T\): anisotropic ratio of the conduction velocity. Data were obtained before (Control) and 30 min after application of 0.5 \(\mu\)mol/L nifekalant (NIF), 10 min after addition of 3 \(\mu\)mol/L lidocaine (NIF+LID), and 15 min after washing lidocaine out (LID wash). The hearts were stimulated at a cycle length of either 400 ms or 250 ms.

Fig 3. Changes in action potential duration (APD) in response to drug application. Left: color-coded maps of APD\(_{90}\) in the recording area were displayed as color gradients in 1.33 ms steps, ranging from red (shortest) to blue (longest). The records were obtained during a constant stimulation at the center (*) of the left ventricular free wall at a cycle length of 400 ms in a heart before (control) and 30 min after application of 0.5 \(\mu\)mol/L nifekalant (NIF), 10 min after addition of 3 \(\mu\)mol/L lidocaine (NIF+LID), and 15 min after washing lidocaine out (LID wash). The traces at the right show optical action potential signals recorded from 4 sites 5 mm away from the center of the left ventricular free wall in the direction of L and T propagation (L1, L2, T1, T2). Averaged APD\(_{90}\) values at the 4 sites and their variation (dispersion) are shown at the bottom. The almost uniform prolongation of APD\(_{90}\) by NIF was partially reversed by addition of LID, and this LID action was abolished after washing out the drug.
5 mm from the stimulation site in direction of L and T propagation (L1, L2, T1, T2). The average APD90 value at the 4 sites increased from 188 ms to 246 ms, but its dispersion was unaffected (7 ms and 9 ms, respectively). The APD prolongation was partially reversed after addition of LID. In the presence of NIF+LID, the average APD90 value returned to 212 ms, but its dispersion was increased to 29 ms. Washout of LID for 20 min resulted in longer APD in the entire area mapped; the average APD90 value at the 4 sites (257 ms) was comparable to the value after application of NIF alone. The dispersion of APD90 was reduced to 18 ms.

The results obtained from 6 hearts at S1–S1 400 ms and 250 ms are summarized in Table 2. At the longer CL (S1–S1 400 ms), application of NIF alone resulted in a significant increase in APD90 (by 36.6±6.8%) without affecting its dispersion. Addition of LID resulted in significant shortening of APD90 (by 14.0±6.4%) and a significant increase in dispersion. After washing out LID, the APD90 and its dispersion returned toward the values after application of NIF alone. Similar but less marked changes in APD90 were induced by application of NIF and LID at the shorter CL (S1–S1 250 ms).
**Induction of VT by Cross-Field Stimulation**

VTs were induced in 6 hearts by S2 cross-field stimulation applied during the vulnerable window of S1 excitation. VTs were polymorphic during the first several beats, but became more stable during the subsequent beats and had an almost monomorphic configuration. Under the drug-free control conditions (control), 81 VT were induced in the S1–S2 interval (vulnerable window) of 130–230 ms. Seventy of the 81 VT terminated spontaneously (non-sustained), whereas the remaining 11 persisted for >30 s (sustained); the incidence of sustained VT was 13.6% (Fig 4A). After application of NIF alone (0.5 μmol/L), VT could also be induced with longer S1–S2 intervals (200–270 ms), but they terminated earlier than under control conditions (Fig 4B). In the presence of NIF alone, all 42 VT terminated spontaneously within 20 beats (non-sustained VT).

**Table 3** Effects of Nifekalant and Lidocaine on the Duration and Cycle Length of Non-Sustained Ventricular Tachycardia (NSVT) Induced by S1-S2 Cross-Field Stimulation

<table>
<thead>
<tr>
<th></th>
<th>n VT duration (s)</th>
<th>VTCL (ms)</th>
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<tbody>
<tr>
<td>Control</td>
<td>70</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>NIF</td>
<td>42</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>NIF + LID</td>
<td>43</td>
<td>2.4±0.9</td>
</tr>
<tr>
<td>LID wash</td>
<td>39</td>
<td>0.8±0.1</td>
</tr>
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</table>

Values are means±SE of 39–70 episodes of NSVT induced in 6 hearts. Data were obtained before (Control) and 30 min after application of 0.5 μmol/L nifekalant (NIF), 10 min after addition of 3 μmol/L lidocaine (NIF + LID), and 15 min after washing lidocaine out (LID wash). Ventricular tachycardias (VTs) were induced by S1-S2 cross-field stimulation. *Significantly different from control at p<0.01. #Significantly different from NIF alone at p<0.05.

**Fig 5.** Modification of spiral dynamics by nifekalant and lidocaine. (Upper panels) isochrone maps of activation during ventricular tachycardias (VT) induced by cross-field stimulation before drug application (A: control), 30 min after application of 0.5 μmol/L nifekalant (B: NIF), and 10 min after addition of 3 μmol/L lidocaine (C: NIF + LID). The data were obtained from the same heart. The isochrones (green lines for the earlier wave fronts, and blue lines for the later wave fronts) were drawn every 4 ms. Thick red lines indicate functional block lines. (Lower panels) Sequential records of optical action potential signals from 9 sites indicated on the respective isochrone map. The cycle of excitation corresponding to the isochrone map (Top) is indicated by the dotted red line, and the next 2 cycles are indicated by dotted blue lines. The cycle length of ventricular excitation (VTCL) of the isochrone map (the cycle indicated by red dotted line in the lower panel) is shown at the bottom. A (control): an almost stable single-loop circuit around a line of functional block is repeated during the VT lasting >30 s. B (NIF): unstable (large meandering) double-loop (figure-8) circuits circulating around 2 lines of functional block are characterized by frequent localized conduction block, giving rise to a shift of the circuits. Ventricular tachycardia terminated within 2 s (third cycle in the lower panel). C (NIF + LID): more stable (little meandering) double-loop (figure-8) circuits circulating around 2 lines of functional block are seen during VT lasting 11 s. Localized conduction block causing a shift of the circuit (third cycle in the lower panel) was less frequent than with NIF alone.
After addition of LID (NIF+LID) the vulnerable window became wider (S1–S2 interval of 170–270 ms), and the VT induced lasted longer than after exposure to NIF alone (Fig 4C). The incidence of sustained VT increased to 5/48 (10.4%). When LID was washed out (LID wash), the vulnerable window shifted again to a similar range as obtained with NIF alone (S1–S2 interval of 180–270 ms), and most of the VT (39/40) terminated spontaneously within 7 beats (Fig 4D).

Table 3 summarizes the CL and the duration of non-sustained VT in the 6 hearts. The non-sustained VT induced in the hearts after exposure to NIF alone were characterized by a significantly longer CL (by 27.1%) and a significantly shorter duration (by 53.7%) than induced under the control conditions. The non-sustained VT induced after additions of LID (NIF+LID) were of significantly longer duration and significantly shorter CL than those induced with NIF alone. The VT with NIF+LID had slightly longer CL than in the controls, but there was no significant difference in VT duration between the 2 groups. Wash-out of LID resulted in a resumption of much shorter duration and much longer CL than those of the control conditions. Thus, the characteristic modification of VT duration and CL by NIF was reversed or minimized by the addition of LID.

Dynamics of Spiral Excitation

Video images of excitation during VT were analyzed in 6 hearts. Under the control conditions some form of rotors (single-loop or figure-8 re-entry) was documented in 11 episodes (61.1%) of the 18 VT available for image analysis. The remaining 7 VT showed one-way propagation of wave fronts traversing the observation area. Stable spirals lasting >5 s were recognized in 6 episodes in 11 VT with visible rotors, whereas in the remaining 5 episodes the rotors were unstable and turned into one-way propagation or were annihilated by collisions with wave fronts from other sites.

Fig 5 shows representative experiments. All data in Fig 5 were obtained from the same heart. Fig 5A illustrates the activation pattern during VT 5 s after the initiation. Counterclockwise rotation of wave fronts around a line of block (~12 mm) can be seen. The VT CL of 5 consecutive beats ranged from 138 ms to 158 ms. The line of block was con-

A local conduction block was recognized between sites 6 and 7, giving rise to a further extension of the functional block line. Site 7 was excited by a wave front turning around the line of the block. The rotor terminated spontaneously 2 cycles later following an extension of the functional block line in the opposite direction (site 4).

Fig 5C shows an activation pattern during a VT of longer duration (lasting for 11 s) after addition of LID (NIF+LID). Figure-8-type double loops circulating around 2 functional block lines were maintained for >5 s with moderate meandering. The VT CL of 5 consecutive beats ranged from 186 to 228 ms. The length of the 2 functional block lines was 14–16 mm and 6–8 mm, respectively. The action potentials recorded from the circuits showed less beat-to-beat variation, and the incidence of local conduction block was reduced.

Qualitatively similar results were obtained in all 6 hearts exhibiting visible rotors under control conditions. Thus, application of NIF alone caused prolongation of the functional block line, an increase in meandering of the circuit, and earlier termination of rotation. All of these effects of NIF were reversed or minimized after addition of LID (NIF+LID).

Discussion

The major findings of the present study are: (1) VT induced in isolated rabbit heart having a 2-D ventricular myocardial structure and uniform anisotropic properties were mediated by spiral-type excitation circulating around variable functional block lines, (2) NIF slowed VT CL and caused early termination due to destabilization of the spiral dynamics, and (3) addition of LID prevented the NIF action on the spiral-type re-entrant VT.

Action Potentials and Conduction Velocity

The anisotropic conduction properties of the 2-D epicardial layer of rabbit ventricles observed in the current study were consistent with those reported by Schalij et al in a similar rabbit heart model with endocardial freezing. In pilot experiments we measured CV and APD on the anterior epicardial surface of the left ventricular free wall in 4 intact rabbit hearts (without endocardial freezing) by using the same stimulation and optical mapping procedures as described in the present paper. Longitudinal and transverse CV ($\overline{\nu}_L$, $\overline{\nu}_T$) in the intact hearts during constant stimulation were 54.6±3.0 cm/s and 23.2±1.5 cm/s, respectively at S1–S1 400 ms, and 55.8±4.0 cm/s and 20.7±1.2 cm/s, respectively at S1–S1 250 ms. The APD90 during the constant stimulation was 179±8 ms at S1–S1 400 ms and 141±5 ms at S1–S1 250 ms. There were no substantial differences between the intact hearts and the 2-D hearts (Tables 1,2) in $\overline{\nu}_L$ and APD90. The transverse CV ($\overline{\nu}_T$) in the intact hearts was slightly faster (by 4–5%) than in the 2-D hearts.
In the present study we used NIF in vitro at a concentration of 0.5 μmol/L, which approximately corresponds to the peak plasma concentration in human subjects (~0.2 mg/L). At this concentration, NIF primarily suppresses the rapid activating component of the delayed rectifier potassium current (IKr). Nifekakant also suppresses other voltage- and ligand-gated potassium channel currents, but at much higher concentrations.

Nifekakant caused nearly uniform prolongation of APD during constant stimulation in our rabbit ventricular muscle preparations, whereas the CV along and across the fiber orientation (caxis and raxis) were unaffected by NIF. These effects can be interpreted by selective block of Ik. Addition of 3 μmol/L LID partially reversed the APD prolongation without affecting the CV, and this action of LID is most likely attributable to blockade of the slow component of Na current (INa, slow) during the plateau-phase action potential. Low concentrations of LID have been shown to suppress INa, slow without affecting INa, fast, which is responsible for the upstroke phase of action potentials, a major determinant of CV in cardiac tissue. The APD of ventricular myocytes is regulated by a delicate balance between inward and outward currents, as the membrane resistance during the plateau phase is very high. Thus, the APD prolongation via reduction of Ik by NIF would be readily counteracted by a concomitant reduction of INa, slow in response to addition of LID.

VTs of Spiral-Type Re-Entry

In the present study we used S1–S2 cross-field stimulation to induce VT of spiral-type excitations; DC field stimulation of moderate intensity (S2: 20 V) was applied during the vulnerable period of preceding excitation (S1) in a direction that crossed the ventricular propagation of S1 excitation. This stimulation protocol is known to most reliably produce a free end of the propagating wave front (wave break), and subsequent curling to produce the tip of the functional block line were actually induced in the observation area in more than half (61%) of all VT episodes under the control conditions. One-way propagation of wave fronts traversing the observation area in the remaining VT episodes (38%) may have arisen from invisible rotors on the opposite surface of the heart. VTs induced were polymorphic during the initial several beats, but stabilized during subsequent beats. This transition is a characteristic behavior of re-entrant VT induced in a 2-D myocardial layer with uniform anisotropy. Discontinuous anisotropic characteristics of the myocardium may set the stage for such anchoring behavior.

VTs induced in the presence of NIF alone are characterized by a longer CL and much earlier spontaneous termination than those observed in the controls. The CL of non-sustained VT was markedly prolonged (by 27%). Such modification was associated with marked destabilization of spiral dynamics documented in the optical images; greater meandering of re-entry circuits, prolongation of functional block lines and frequent occurrence of local conduction blocks.

The NIF-induced modification of spiral dynamics may be the result of a repolarization delay in the circuit. All of the action potentials in the re-entrant circuit in the spiral-type excitations during VT in the absence (control) and presence of NIF were elicited successively, with minimal isoelectric segments between excitations. The fluorescence signals do not represent absolute transmembrane potential, and their precise interpretation is hampered by low-pass space filtering. Despite such limitations, however, the action potential signal configurations around the functional block line seem to suggest minimal electrical diastole reflecting an excitatory gap in the circuit. Substantial prolongation of APD in such re-entrant circuits should lead to a tremendous increase in interactions between the wave-front and wave-tail. The functional block line, which is formed by the refractory wake of the wave moving in the opposite direction, has to be prolonged to maintain the rotation. The wave front would encounter its own tail more and more frequently and produce numerous local conduction blocks, giving rise to the complex meandering of the circuit. This may increase the opportunity for breakup or annihilation of the rotation.

The NIF-induced changes in VT duration, VT CL, and spiral dynamics were all partially reversed by addition of LID. This LID-induced reversal is interpreted as most likely being due to a counteracting abbreviation of APD.

Clinical Implications

In the present study, NIF and LID were applied at the beginning in the conditioning period of constant stimulation (before induction of VT). However, since in the clinical practice these drugs are usually used in patients with VT/VF, this difference in the drug application mode should be kept in mind when considering the clinical implications of our data.

In many recent model and experimental studies the investigators have postulated that spiral-type excitations (rotors) are the central organizing machinery of VF. Two major mechanisms for the maintenance of VF have been identified. Some investigators have proposed that VF results from the instability of rotors, which ultimately leads to their continuous breakup, while others have hypothesized that fibrillation is maintained by wave fronts emanating at an exceedingly high frequency from a relatively stable rotor. In other words, a rapidly circulating rotor (mother rotor) is the fundamental driver of VF, and the multiple wavelets that characterize VF are epiphenomena caused by fibrillatory conduction block. These mechanisms are not totally incompatible, but may make complementary contributions in various heart diseases.

Based on the results of the present study, NIF is expected to be effective in preventing VF, if the VF is attributable to rapidly circulating mother rotors, as it destabilizes rotation activity. Failure of ventricular defibrillation by DC shocks at an energy below a certain level is mainly attributable to induction of new mother rotor-type excitations, giving rise to re-initiation of fibrillation. Administration of NIF would be effective in preventing such shock-induced re-initiation of fibrillation. In canine experiments, in fact, NIF has been shown to lower the defibrillation threshold. Addition of LID would antagonize these beneficial actions of NIF.

However, NIF has the ability to increase VF perpetuation, if it is due to continuous breakup of wave fronts. Addition of LID might antagonize such an undesirable action.
of NIF. More extensive experimental studies are required to resolve these issues.

Study Limitations
In the current study we used a 2-D subepicardial layer of ventricular myocardium to analyze drug actions on spiral-type re-entrant arrhythmias, but the dynamics of spiral-type excitations (scrolls) in real hearts, with their 3-D structure, are much more complex. Moreover, spontaneous VT/VF normally occurs in diseased hearts with a variety of functional and morphological abnormalities, not in healthy hearts. We used BDM as an excitation-contraction uncoupler, but it is known to have substantial effects on the electrophysiological characteristics of cardiac myocytes. Moreover, there are considerable species differences in the relative contribution of the rapidly and slowly activating components of the delayed rectifier potassium current (IKr and IKs) to the repolarization of action potentials in ventricular myocytes. The relative contribution of IKs has been shown to be much greater in rabbits than in other species (guinea pigs, dogs, and perhaps humans). The effects of NIF on the spiral-type re-entrant VT in humans may therefore be less prominent than in rabbits. These limitations should be taken into account in the application of the observations in the present study to the treatment of VT/VF in clinical practice.

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