Therapeutic Hypothermia (30°C) Enhances Arrhythmogenic Substrates, Including Spatially Discordant Alternans, and Facilitates Pacing-Induced Ventricular Fibrillation in Isolated Rabbit Hearts

Yu-Cheng Hsieh, MD; Shien-Fong Lin, PhD*; Tung-Chao Lin, MD; Chih-Tai Ting, MD, PhD; Tsu-Juey Wu, MD, PhD

Background: Therapeutic hypothermia (TH, 30°C) protects the brain from hypoxic injury. However, TH may potentiate the occurrence of lethal ventricular fibrillation (VF), although the mechanism remains unclear. The present study explored the hypothesis that TH enhances wavebreaks during VF and Si pacing, facilitates pacing-induced spatially discordant alternans (SDA), and increases the vulnerability of pacing-induced VF.

Methods and Results: Using an optical mapping system, epicardial activations of VF were studied in 7 Langendorff-perfused isolated rabbit hearts at baseline (37°C), TH (30°C), and rewarming (37°C). Action potential duration (APD)/conduction velocity (CV) restitution and APD alternans (n=6 hearts) were determined by Si pacing at these 3 stages. During TH, there was a higher percentage of VF duration containing epicardial repetitive activities (spatiotemporal periodicity) (P<0.001). However, TH increased phase singularity number (wavebreaks) during VF (P<0.05) and Si pacing (P<0.05). TH resulted in earlier onset of APD alternans (P<0.001), which was predominantly SDA (P<0.05), and increased pacing-induced VF episodes (P<0.05). TH also decreased CV, shortened wavelength, and enhanced APD dispersion and the spatial heterogeneity of CV restitution.

Conclusions: TH (30°C) increased the vulnerability of pacing-induced VF by (1) facilitating wavebreaks during VF and Si pacing, and (2) enhancing proarrhythmic electrophysiological parameters, including promoting earlier onset of APD alternans (predominantly SDA) during Si pacing. (Circ J 2009; 73: 2214–2222)

Key Words: Cardiac alternans; Hypothermia; Optical mapping; Restitution; Ventricular fibrillation

Therapeutic hypothermia (TH, 30°C) has decreased intracranial pressure, slowed cerebral metabolic processes, and protected the brain from anoxic injury in animal models and human scenarios.1–3 Recent guidelines also recommend that unconscious adult patients with spontaneous circulation after out-of-hospital ventricular fibrillation (VF) cardiac arrest should be cooled to 32–34°C for 12–24h.4–6 Although previous studies showed that extreme hypothermia (<29°C) might potentiate the occurrence of lethal ventricular arrhythmia,7–9 the myocardial substrate properties in the therapeutic range of hypothermia (≥30°C) are not completely understood. Because 30°C is the lowest temperature that has proven to be feasible in clinical practice,1–3 we chose it to test the ventricular substrate for arrhythmogenesis in the present study.

One mechanism for determining the maintenance of VF is its wavefront characteristics during VF.10,11 Harada et al found that in 2-dimensional rabbit ventricular preparations, TH (30°C) enhanced wavebreaks and regeneration of new spiral waves during VF, facilitating the maintenance of ventricular tachycardia (VT) and VF.12 While using a clinically appropriate temperature of 32–34°C, they demonstrated that the spiral waves of VT/VF frequently collided and dissipated in favor of self-termination of VT/VF, a different effect from that of TH at 30°C.12 Whether the wavefront characteristics (ie, wavebreaks) of VF in this 2-dimensional model also occur in 3-dimensional intact hearts at TH (30°C) remains unclear. Cardiac alternans, particularly spatially discordant alternans (SDA), is a key arrhythmogenic factor for ventricular tachyarrhythmia.13 However, limited information is available regarding cardiac alternans properties during TH (30°C).14

In this study, using an optical mapping system, we investigated the wavefront characteristics of VF (ie, wavebreaks, spatiotemporal periodicities), cardiac electrophysiological/alternans properties, and the vulnerability of pacing-induced VF specifically at TH (30°C) in 3-dimensional Langendorff-perfused isolated rabbit hearts. We hypothesized that TH (30°C) enhances wavebreaks during VF and Si pacing, facilitates pacing-induced SDA, and increases the vulnerability of pacing-induced VF.
Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital.

Langendorff Preparation and Pseudo-ECG Recordings

The hearts of New Zealand white rabbits (2.8–3.9 kg) were excised under general anesthesia. The ascending aorta was cannulated and perfused with 37°C Tyrode’s solution composed of (in mmol/L): 125 NaCl, 4.5 KCl, 0.5 MgCl₂, 24 NaHCO₃, 1.8 NaH₂PO₄, 1.8 CaCl₂, 5.5 glucose, and albumin (40 mg/L). The coronary perfusion pressure and flow rate were 60–65 mmHg and 35–45 ml/min, respectively. Next, the hearts were perfused and superfused in a thermostatted tissue bath. A pseudo-ECG was obtained with widely spaced bipoles to determine ventricular rhythm. The signals were digitized by an AxoScope with a sampling rate of 1 kHz.

Optical Mapping

Using a 2-camera optical mapping system, epicardial activations in the anterior and posterior aspects of the hearts were simultaneously mapped. The hearts were stained with di-4-ANEPPS, and excited with 4 light-emitting diode modules (wavelength=519±20 nm). Induced fluorescence was collected by 2 image-intensified charge-coupled cameras (model CA DI-0128T). Optical signals were gathered at 3.85 ms sampling intervals, acquired from 128×128 sites simultaneously over a 30×30-mm² area in each aspect of the heart. For each recording, optical data were acquired continuously for 3.85 s (1,000 frames/phase maps). In a typical time-embedded phase portrait, the upstroke of the action potential corresponds to a phase ranging from −3/4 to −1/4 π, roughly the light-blue color (between dark-blue and green) using color representation. Phase mapping was performed to evaluate the wavefront characteristics, and the location and evolution of phase singularities (PSs). A PS shown on the phase maps was defined as a site with an ambiguous phase surrounded by pixels exhibiting a continuous phase progression from −π to +π. Previous studies suggest that PSs are a robust alternate representation of wavebreaks, which serve as the source of VF.

Induction of Hypothermia (30°C) and Rewarming (37°C)

Two thermostatic systems (37°C and 30°C) were connected in parallel to the Langendorff system. By controlling a switch between them, the temperature of the perfusate and tissue bath could be switched to either 37°C or 30°C. At baseline, the temperature of both media was maintained at 37°C. To induce hypothermia, we switched the thermostatic system to 30°C and the superfusate was also quickly replaced with 30°C Tyrode’s solution. During the cooling procedure, the temperature in the upper, middle, and lower thirds of the tissue bath was checked every 1–2 min until 30°C was achieved at all levels. When the tissue bath temperature reached 30°C, an additional 5-min cooling (stabilized at 30°C) was used to ensure the homogeneity of tissue temperature, and then the study protocol was started.

To re-warm (37°C) the heart, the procedure was reversed. In this study, it took 5–7 min to complete the cooling or re-warming procedure.

Study Protocols

Protocol I: Wavefront Characteristics During Different Stages of VF–Baseline (37°C), Hypothermia (30°C), and Rewarming (37°C) (n=7) We used burst pacing to induce VF at baseline. Baseline VF was defined as a stable VF that persisted for 5 min after pacing induction. Three sets of optical data and corresponding pseudo-ECG recordings were obtained during baseline VF, then the hearts were cooled to 30°C. After the cooling procedure, optical and pseudo-ECG recordings of VF were obtained at 1, 5, 10, and 15 min during hypothermia. Next, the heart was re-warmed to 37°C and after completion of the re-warming procedure, recordings were again obtained at 1, 5, and 10 min. When VF terminated during the 15-min hypothermia or the 10-min re-warming period, it was immediately re-induced by burst pacing.

Protocol II: Si Pacing at Baseline (37°C), 5-min Hypothermia (30°C), and 5-min Rewarming (37°C) (n=6) Si pacing (2×diastolic threshold) was used to determine the action potential duration (APD)/conduction velocity (CV) restitutions, cardiac alternans properties, and vulnerability of pacing-induced VF at baseline, 5-min hypothermia and 5-min rewarming. To estimate CV, the inverse conduction time (CT⁻¹, cm/s) between 2 epicardial points was measured (Figure 1B in Wu et al). APD and CT⁻¹ restitutions were determined using 12 different Si pacing cycle lengths (PCLs). For each PCL, an Si pacing train was delivered for 15 s, and optical data were recorded at the end of the pacing train. If VF was induced and persisted for >1 min after stopping Si pacing, a defibrillation shock was delivered through a defibrillation coil. To minimize motion artifacts, cytochalasin-D (5 μmol/L), an excitation–contraction uncoupler, was used.

Data Analysis

Fast Fourier Transforms (FFTs) Analysis and Epicardial Wavefront Characteristics During VF

Pseudo-ECGs (4 s duration) were used to determine the dominant frequency (DF) at 8 different time points of VF in protocol I: baseline, 1-, 5-, 10-, 15-min hypothermia, and 1-, 5-, 10-min rewarming. Similarly, the epicardial wavefront characteristics of VF were analyzed at the same 8 time points. At each time point, one optical recording was analyzed in each heart studied.

By displaying the optical recording frame-by-frame, epicardial repetitive activities (ERAs) with lifespan ≥2 activations were identified and analyzed. ERAs were defined as an epicardial breakthrough, an epicardial reentrant wavefront or a large wavefront arising outside mapped regions. To quantify wavebreaks during VF, the number of PSs in the phase map were counted manually every 5 frames for 1,000 frames in each optical recording studied.

Construction of APD and CT⁻¹ Restitution Curves Using Si Pacing Method

The method of constructing APD and CT⁻¹ restitution curves has been reported elsewhere. Briefly, pixels at the center of the anterior and posterior surfaces of both ventricles (sites measured in the Langendorff preparation) were selected to determine the APD restitutions (APD at 70% repolarization). APD restitution (APDR) curves of each heart were plotted with means of APD restitutions from the 4 sites against different Si PCLs. When APD alternans occurred during Si pacing, the short and long APD restitutions were averaged. CT⁻¹ restitution of each heart was plotted with means of CT⁻¹ along 4 evenly distributed epicardial lines (Figure 1B in Wu et al) against different Si PCLs. With the formula for restitution, the APD and CT⁻¹ restitution curves were obtained.
APD\(70\times\text{CT}^{-1}\) = WL (wavelength, cm). WL restitution were obtained. The method for determining the maximal slope of the APDR has been previously described.\(^{18}\) The maximum slope of the APDR for each heart is the mean of the maximum APDR slopes of the 4 sampling sites.

**APD Alternans and Wavebreaks During S\(_1\) Pacing**

APD alternans was defined as the difference in APD\(70\) of 2 consecutive beats of \(\geq 3.85\) ms during S\(_1\) pacing.\(^{23}\) The alternans threshold was defined as the longest S\(_1\) PCL at which APD alternans was detected.\(^{23–25}\) Spatially concordant alternans (SCA) was defined as APD alternation in phase spatially (ie, for a given beat, the APD is either long or short everywhere in the tissue), whereas SDA was defined as alternation out of phase spatially (ie, some regions of tissue alternate in a long-short-long pattern, while other regions simultaneously alternate in a short-long-short pattern).\(^{13,23}\)

To determine the presence of SCA or SDA during APD alternans, APD difference maps were created from the difference in APD\(70\) between 2 consecutive beats.\(^{23,26}\) APD difference maps were shaded red if the differences were positive, and green if negative.\(^{23}\) Thus, during SCA, APD difference maps showed all green on 1 beat and all red on the next beat. During SDA, red and green regions alternated, separated by a nodal line (NL, shown in white) in which no alternans was present.\(^{23}\) By analyzing the APD difference maps during SDA, the average number of NLs at each PCL was obtained. During S\(_1\) pacing, the average number of PSs in each phase map was counted manually every 5 frames for 1,000 frames in each optical recording studied.

**Statistical Analysis**

Data are presented as mean±SD. Paired and unpaired t-tests, and chi-square analysis with Yates correction were used to compare the data between and within groups. A probability value of P\(<0.05\) was considered significant.

**Results**

**Protocol I**

**FFT Analysis of Pseudo-ECG During Different Stages of VF** Baseline VF was successfully induced in all 7 hearts. The mean DF of VF decreased during hypothermia (Table 1).
Wavefront Characteristics During Different Stages of VF  A total of 56 optical recordings were selected for analysis from all 7 hearts studied at 8 different time points of VF.

Occurrence of ERAs  In these 56 optical recordings, a total of 450 runs of ERAs were identified (Table 1). At baseline VF, optical data showed multiple wandering wavelets with occasional occurrence of ERAs. During hypothermia, the average runs of ERAs in each recording window significantly increased (Table 1). In the 8 time points, most ERAs showed as epicardial breakthrough pattern (Table 1). Although the mean lifespan (in activations) of these ERAs during hypothermia did not differ from baseline, the mean activation interval (ie, cycle length) of these ERAs increased during hypothermia (Table 1). Therefore, the percentage of recording time containing ERAs significantly increased during hypothermia (Table 1).

Number of PSs  At baseline VF, the average number of PSs in each phase map was 6.2±0.9 (Figure 1B). During hypothermia, optical data showed frequent wavebreaks while the average number of PSs increased (8.3±1.1, 8.2±1.2, 7.6±1.0, and 7.9±0.7 for 1-, 5-, 10-, and 15-min hypothermia, respectively; P<0.05 for all when compared with baseline) (Figure 1B). At 10-min rewarming, the number of PSs returned to the baseline level (6.9±0.7*, 6.8±0.8*, and 6.6±0.9 for 1-, 5-, and 10-min rewarming, respectively; *indicating P<0.05 compared with baseline). Figures 1C–E is an example.

Protocol II  One episode of spontaneous VF was observed 7 min after the initiation of the cooling process (heart no. 4), but no tachyarrhythmia was observed during the rewarming of the 6 hearts.

Effects of Hypothermia (30°C)  The effects of hypothermia on APD, CT−1, and WL restitutions are sum-
Table 2. Effect of Therapeutic Hypothermia (30°C) on APD₀, CT⁻¹ and WL

<table>
<thead>
<tr>
<th>PCL, ms</th>
<th>APD₀, ms</th>
<th>CT⁻¹, cm/s</th>
<th>WL, cm</th>
</tr>
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<td>400</td>
<td>157±18</td>
<td>0.9±0.9</td>
<td>3.7±1.1</td>
</tr>
<tr>
<td>350</td>
<td>153±14</td>
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<td>3.7±1.1</td>
</tr>
<tr>
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<td>3.5±1.0</td>
</tr>
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<td>1.2±1.2</td>
<td>3.3±1.0</td>
</tr>
<tr>
<td>200</td>
<td>127±8</td>
<td>1.3±1.3</td>
<td>3.1±1.0</td>
</tr>
</tbody>
</table>

*Data from hearts 2 and 3 of Protocol II.
**Data from hearts 1, 3, and 4 of Protocol II.
†P<0.05, ‡P<0.01, by paired t-test when compared with baseline.

Figure 2A shows an example: during hypothermia, APD₀ was prolonged, shifting the APDR curve upwards (Figure 2Aa), while CT⁻¹ decreased (Figure 2Ab). WLs were shorter than those at baseline with short PCLS (≤250ms) (Figure 2Ac).

The maximum slope of the APDR during hypothermia (1.8±0.21) was similar to that at baseline (1.5±0.32) or rewarming (1.7±0.13) (P=0.11). Figure 2B shows an example.

APD dispersion and spatial heterogeneity of restitutions

APD₀ dispersion (difference between the maximum and minimum APD₀ from entire mapped area) significantly increased during hypothermia (Figure 3A). APD₀ dispersion at 5 PCL of 300 ms increased during hypothermia (37±5 ms) compared with that at baseline (20±6 ms) and rewarming (23±5 ms) (P<0.001). Similarly, APD₀ dispersion at 5 PCL of 250 ms (P<0.001) and 200 ms (P<0.002) also increased during hypothermia.

“Maximum CT⁻¹ reduction” was used to estimate the spatial heterogeneity of CT⁻¹ restitution (cm/s) (see Figure 6B in Wu et al18). There was no difference in maximum CT⁻¹ reduction along the 4 epicardial lines at baseline (15±6, 11±4, 9±7, 11±9 cm/s, lines 1–4, respectively, P=0.556) and rewarming (19±8, 14±5, 8±6, 13±11 cm/s, lines 1–4, respectively, P=0.089) (Figure 3B). However, the heterogeneity significantly increased during hypothermia (12±7, 7±5, 4±2, 6±4 cm/s, lines 1–4, respectively, P=0.015) (Figure 3B).
At baseline, the maximum slope of the APDR was similar among all 4 recording sites (1.62±0.52, 1.55±0.34, 1.54±0.35, 1.52±0.36, sites a–d, respectively P=0.976). This heterogeneity remained insignificant during hypothermia (P=0.718) and rewarming (P=0.662).

Effects of Hypothermia (30°C) on Pacing-Induced APD Alternans, Wavebreaks and VF

Figure 4 shows an example of the effect of hypothermia on alternans properties. Compared with baseline (146±16 ms) and rewarming (153±14 ms), the alternans threshold was significantly prolonged during hypothermia (292±38 ms, P<0.001) (Figure 4E). At the alternans threshold, SDA was observed in 4 hearts during hypothermia, compared with 0 and 1 heart at baseline and rewarming, respectively (P=0.027). At PCL of 250 ms, the average number of NLs (0, 2.0±0.6, 0, for baseline, hypothermia and rewarming, respectively, P<0.001) increased during hypothermia (Figure 4F). Similarly, the average number of NLs also increased during hypothermia at S1 PCLs of 200 ms (P<0.001) and 180 ms (P=0.005) (Figure 4F).

During S1 pacing, VF was not inducible at baseline or rewarming at all S1 PCLs in all 6 hearts studied. However, during hypothermia, 6 sustained VF episodes were induced in 3 hearts at PCL ≤200 ms when S1 pacing trains were stopped (P=0.027). In 5 of these 6 episodes, SDA was observed during S1 pacing immediately before the initiation of VF, and in the 6th there was SCA before VF initiation. Figure 5 shows an example of S1 pacing-induced VF at S1 PCL of 180 ms. During hypothermia (Figure 5B), S1 pacing induced SDA (first 4 beats in Figure 5Bc), which was immediately followed by VF-like activations (Figure 5Bc). The average number of PSs at S1 PCL of 200 ms increased

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**Figure 4.** Phase maps, local optical recordings, and APD difference maps at alternans threshold during (A) baseline (PCL: 140 ms), (B, C) hypothermia (B, PCL: 250 ms at alternans threshold; C, PCL: 180 ms), and (D) rewarming (PCL: 160 ms). Data from heart no. 4 of Protocol II. At baseline, a snapshot of phase map (Aa) shows the pacing wavefront. Local optical recordings at sites x and y demonstrate concordant alternans (Ab). APD difference maps show all green for beats 1–2 (Ac) and all red for beats 2–3 (Ad), suggesting the entire tissue alternans is in phase. During hypothermia, sites x and y demonstrated discordant alternans (Bb, Cb). APD difference maps show 2 areas (green and red) alternans out of phase, separated by white nodal lines (NLs, dotted lines) (Bc–d, Cc–d). Note that as the PCL further decreased to 180 ms during hypothermia (C), the number of NLs increased (Cc–d), and APD difference maps became more complicated (Cc–d). During rewarming (Dc–d), the alternans properties were similar to those at baseline. (E) The average alternans threshold during the 3 stages (baseline, hypothermia, rewarming). The P value was obtained by ANOVA. (F) The average number of NLs in APD difference maps at S1 PCL of 300, 250, 200 and 180 ms in the 3 stages. The P values were obtained by ANOVA at each PCL. Note absence of NL at baseline or during rewarming. See text for abbreviations.
during hypothermia (0.60±0.15), compared with baseline (0.30±0.10) and rewarming (0.44±0.21) (P=0.023). Similarly, the average number of PSs at S1 PCL of 180 ms also increased during hypothermia (P<0.001).

Discussion

This study has the following major findings. TH at 30°C (1) facilitates the occurrence of ERAs (ie, increase in spatiotemporal periodicity) during VF, but increases the PS number (ie, wavebreaks) during VF and S1 pacing; (2) enhances proarrhythmic parameters, by decreasing CV, shortening WL, and enhancing APD dispersion and the spatial heterogeneity of CT–1 restitution (ie, CV restitution); (3) results in earlier onset of APD alternans (ie, the alternans threshold is prolonged), which is predominantly SDA; and (4) increases the vulnerability of pacing-induced VF. We specifically used TH at 30°C (below the clinically appropriate temperature) in the present study, and we found that this temperature caused ventricular arrhythmias and the possible mechanisms were analyzed.

Wavefront Characteristics of VF During TH (30°C)

Interventions that increase the spatial coherence and temporal regularity of VF may hinder the maintenance of VF. Chorro et al reported that in isolated rabbit hearts, acute reduction of ventricular temperature to <20°C spontaneously terminated VF, which was associated with simplified VF activation patterns. In the present study using TH (30°C), we observed that the percentage of recording time containing ERAs (spatiotemporal regularity of VF) increased during VF, which has not previously been reported. In contrast, the average number of PSs during VF significantly increased, suggesting frequent wavebreaks during VF and favoring the maintenance of VF. Similarly, using a 2-dimensional ventricular preparation, Harada et al reported that spiral wave excitations of VT/VF were associated with frequent wavebreaks (increased PSs) and an increased incidence of direct current stimulation induced VT/VF during TH (30°C).
Electrophysiological Properties During TH (30°C)

Electrophysiological parameters determining a predisposition to ventricular tachyarrhythmia include: dispersion of repolarization/refractoriness, slow conduction, heterogeneity of CV, shortened WL and restitution properties. Fedorov et al reported that hypothermia at 27°C and 17°C results in non-uniform conduction slowing, increased dispersion of repolarization, and shortened WL in isolated rabbit hearts. Using a clinically applicable temperature (30°C), we observed that TH decreased CV, shortened WL, and enhanced APD dispersion and spatial heterogeneity of CT restitution, consistent with the results reported by Harada et al in a 2-dimensional rabbit ventricle at TH (30°C). The only difference was that Harada et al reported that the maximal slope of APDR significantly increased, whereas we found no difference among baseline, TH (30°C) and rewarming.

Cardiac Alternans During TH (30°C)

Cardiac alternans may be SCA, when all regions of tissue alternate in phase, or SDA, when adjacent regions alternate out of phase, separated by a NL at which no alternans occurs. Typically, SDA occurs at higher temperatures than SCA and is associated with increased vulnerability of pacing-induced VF based on the following evidence. Firstly, most (5 of 6) VF episodes induced by SI pacing predominantly induced SDA during TH (30°C), whereas it was SCA at baseline and during rewarming. These SDA properties were associated with increased vulnerability of SI pacing-induced VF during TH (30°C). We believe that SDA might contribute to the increased vulnerability of pacing-induced VF based on the following evidence. Firstly, most (5 of 6) VF episodes induced by SI pacing were preceded by SDA and frequent wavebreaks before the initiation of VF during TH (30°C) (Figure 5B). Secondly, TH (30°C) significantly increased the number of NLs (Figure 4F), at which localized conduction block and reentry arrhythmia are reported to occur frequently. The mechanisms by which SI pacing induced predominantly SDA during TH (30°C), whereas it was SCA at baseline and during rewarming, to functional conduction block and subsequent occurrence of SDA during TH (30°C). Furthermore, the maximum slope of the APDR (1.86±0.21) during TH (30°C) remained >1, which might also contribute to the occurrence of SDA.

Proarrhythmic Parameters and Wavebreaks During TH (30°C)

Proarrhythmic parameters, such as CV slowing, the spatial heterogeneity of CV restitution, dispersion of repolarization, steep APDR, shortened WL, and SDA, have all been documented as causing conduction block and wavebreaks, and facilitating the initiation of arrhythmia. We also observed that the enhancement of these proarrhythmic parameters during TH (30°C) was associated with an increased number of PSs (ie, wavebreaks) during SI pacing and VF. These findings suggest that these accumulated proarrhythmic parameters during TH (30°C), either via a single dominant factor (such as SDA) or by a synergistic effect of multiple coexisting factors, contribute to the occurrence of wavebreaks during SI pacing, leading to increased vulnerability to pacing-induced VF. However, because these dynamic proarrhythmic factors are simultaneously observed during TH (30°C), it was difficult to determine their individual importance in producing wavebreaks in the present study.

Clinical Implications

If bradycardia or heart block necessitating ventricular pacing is considered in patients undergoing TH (30°C), the ventricular pacing rate should be carefully determined in order not to induce cardiac alternans. Furthermore, placement of catheters or temporary ventricular leads could induce ectopic beats or short VT runs, which could in turn degenerate to VF, and should be performed with extreme caution. Because the temperature of 32–34°C preserved antiarrhythmic effects in favor of self-termination of VT/VF, cooling to 30°C may become arrhythmogenic when compared with 32–34°C.

Study Limitations

In most (5 of 6) of the SI pacing-induced VF episodes, mapping data at the exact initiation of VF were not available. Therefore, the mechanisms of the SDA shift to VF was not completely verified in this study. Secondly, we immersed the entire heart in a thermostatized tissue bath. After the cooling (or rewarming) procedure, an additional 5-min period with a stabilized target temperature was used to ensure the homogeneity of tissue temperature. It is unlikely that the epicardium and endocardium of the heart would have developed significant temperature differences during the experiment. However, we did not directly measure the transmural temperature distribution throughout the heart. Thirdly, cytochalasin-D was only used in Protocol II, and not in Protocol I. Whether or not the electrophysiological properties obtained in Protocol II could be fully extrapolated to VF activations in Protocol I remains to be investigated. Finally, optical mapping data were recorded from the epicardial surface. Whether or not intramural activations were altered during TH (30°C) requires further study.

Conclusion

We report that the wavefront characteristics of VF are associated with frequent wavebreaks during TH (30°C) in Langendorff-perfused intact rabbit hearts. In addition to
demonstrating enhanced proarrhythmic electrophysiological parameters, we elucidated that SDA with NL formation was frequently observed at S1 pacing during TH (30°C). Cardiac alternans properties during TH (30°C) might also play a role in the increased vulnerability to pacing-induced VF and wavebreaks. These findings strongly suggest that at 30°C, TH creates arrhythmogenic substrates that enhance both the initiation (pacing-induced SDA and wavebreaks) and maintenance (frequent wavebreaks during VF) of VF, and therefore TH at this temperature should be carefully implemented.

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