

Enhanced Dispersion of Repolarization Explains Increased Arrhythmogenesis in Severe Versus Therapeutic Hypothermia

Joseph S. Piktel, MD; Darwin Jeyaraj, MD; Tamer H. Said, MD;
David S. Rosenbaum, MD; Lance D. Wilson, MD

Background—Hypothermia is proarrhythmic, and, as the use of therapeutic hypothermia (TH) increases, it is critically important to understand the electrophysiological effects of hypothermia on cardiac myocytes and arrhythmia substrates. We tested the hypothesis that hypothermia-enhanced transmural dispersion of repolarization (DOR) is a mechanism of arrhythmogenesis in hypothermia. In addition, we investigated whether the degree of hypothermia, the rate of temperature change, and cooling versus rewarming would alter hypothermia-induced arrhythmia substrates.

Methods and Results—Optical action potentials were recorded from cells spanning the transmural wall of canine left ventricular wedge preparations at baseline (36°C), during cooling and during rewarming. Electrophysiological parameters were examined while varying the depth of hypothermia. On cooling to 26°C, DOR increased from 26±4 ms to 93±18 ms ($P=0.021$); conduction velocity decreased from 35±5 cm/s to 22±5 cm/s ($P=0.010$). On rewarming to 36°C, DOR remained prolonged, whereas conduction velocity returned to baseline. Conduction block and reentry was observed in all severe hypothermia preparations. Ventricular fibrillation/ventricular tachycardia was seen more during rewarming (4/5) versus cooling (2/6). In TH ($n=7$), cooling to 32°C mildly increased DOR (31±6 to 50±9, $P=0.012$), with return to baseline on rewarming and was associated with decreased arrhythmia susceptibility. Increased rate of cooling did not further enhance DOR or arrhythmogenesis.

Conclusions—Hypothermia amplifies DOR and is a mechanism for arrhythmogenesis. DOR is directly dependent on the depth of cooling and rewarming. This provides insight into the clinical observation of a low incidence of arrhythmias in TH and has implications for protocols for the clinical application of TH. (*Circ Arrhythm Electrophysiol.* 2011;4:79-86.)

Key Words: hypothermia ■ dispersion of repolarization ■ arrhythmias ■ ventricular fibrillation ■ optical mapping

Therapeutic hypothermia (TH) is a recommended therapy for patients who have cardiac arrest caused by ventricular fibrillation (VF) and have subsequent return of spontaneous circulation as it improves neurological function and mortality.¹⁻⁴ However, hypothermia is also proarrhythmic. Accidental hypothermia accounts for approximately 700 deaths per year.⁵ Arrhythmias are encountered in the majority of patients with severe hypothermia (<30°C), including VF that can be refractory to standard therapy.⁶⁻⁸ Although arrhythmias are common in severe hypothermia, an increased susceptibility to arrhythmias was not observed in postresuscitation patients treated with TH.^{1,2} The reason for the relatively low incidence of arrhythmias in patients undergoing TH is unknown.

Clinical Perspective on p 86

The mechanisms underlying arrhythmogenesis in hypothermia remain unclear. Hypothermia has well-known effects

on ventricular repolarization, prolonging action potential duration (APD) and resulting in prolongation of the QT interval.^{9,10} Multiple sarcolemmal ionic currents that govern repolarization are particularly susceptible to hypothermia (exp, I_{to} , I_{CaL} , NCX, I_{Kr}).¹¹⁻¹⁹ Importantly, hypothermia enhances heterogeneities of repolarization.²⁰ Enhanced heterogeneities of repolarization are associated with increased susceptibility to reentrant excitation and arrhythmogenesis.²¹ In particular, disease-induced enhancement of the electrophysiological heterogeneities between the transmural cell types of ventricle are mechanistically related to genesis of arrhythmias.^{22,23} Because there is heterogeneous transmural expression of temperature-dependent sarcolemmal membrane currents, it is likely that hypothermia-induced transmural dispersion of repolarization (DOR) may be important in the mechanism of hypothermia-induced arrhythmogenesis.²⁴ Given the increasing use of TH and

Received July 8, 2010; accepted November 22, 2010.

From The Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, OH.

Presented in part at the Scientific Sessions of the American Heart Association, Chicago, IL, 2006, and Society of Academic Emergency Meeting Annual Meeting, Washington, DC, 2008.

Correspondence to Joseph S. Piktel, MD, MetroHealth Campus, Case Western Reserve University, 2500 MetroHealth Dr, Cleveland, OH 44109-1998. E-mail jpiktel@metrohealth.org

© 2011 American Heart Association, Inc.

Circ Arrhythm Electrophysiol is available at <http://circep.ahajournals.org>

DOI: 10.1161/CIRCEP.110.958355

development of technologies for rapid cooling and rewarming, particularly with the known proarrhythmic effects of severe hypothermia, it is important to understand the electrophysiological effects of hypothermia on cardiac myocytes and arrhythmia substrates.

We used a previously developed model for studying transmural heterogeneities of repolarization, the canine ventricular wedge preparation, to test the hypothesis that hypothermia-enhanced transmural DOR is a mechanism of arrhythmogenesis in hypothermia. In addition, we investigated whether the degree of hypothermia, the rate of temperature change, and the effects of cooling versus rewarming might alter hypothermia-induced arrhythmia substrates.

Methods

Optical Mapping in the Canine Wedge Preparation

Experiments were carried out in accordance with Public Health Service guidelines for the care and use of laboratory animals. The details of our system for high-resolution transmural optical mapping of the arterial perfused canine wedge preparation were described previously.^{25–27} Briefly, wedges of myocardium harvested from the left ventricle were isolated and perfused. Wedges were then placed in an imaging chamber, a water bath with a clear vertical surface for imaging. The wedge was then placed in the chamber with the transmural surface against the glass imaging plate to allow for excitation and signal transduction. The wedges were optically mapped to assess electrophysiological properties of all cell types spanning the transmural wall. The wedge was perfused with a voltage-sensitive dye (di-4-ANNEPS, 15 $\mu\text{mol/L}$). Action potentials were recorded from 256 sites simultaneously with high spatial (0.89 mm), temporal (0.5 ms), and voltage (0.5 mV) resolution. Optical magnification of $\times 1$ to 1.2 was used. Cytochalasin D (6 μM) was used to eliminate motion artifact from the optical signals.^{25–27} Perfusion pressure was maintained between 50 to 60 mm Hg. Preparations remained stable for at least 4 hours,²⁷ with no changes in CV or DOR, based on time control studies. A validation study was also performed to ensure that the cytochalasin D did not affect DOR and arrhythmogenesis during hypothermia. There were no significant differences in CV, DOR, APD, or arrhythmia inducibility between preparations in which cytochalasin D was used when compared with the validation set without cytochalasin-D.

Temperature Manipulation and Hypothermia Protocols

The imaging chamber was encased in a water insulated circuit, which allowed for precise temperature manipulation. Temperature was measured using a digital temperature probe (Omega) in the water bath, allowing for temperature precision of 0.1°C. Care was taken to measure temperature at multiple sites along the transmural surface to ensure homogeneous transmural temperature in the preparations throughout the experiments. A subset of experiments was performed to detect time- and sight-dependent differences in temperature throughout cooling and rewarming. Temperature was measured in the (1) water bath, (2) epicardial surface of the imaging window, (3) endocardial surface of the imaging window, and (4) middle of the wedge on the imaging window, and these data were compared. There was minimal difference across the transmural surface of the imaging window throughout cooling and rewarming ($0.1 \pm 0.02^\circ\text{C}$). There was also minimal difference between the water bath and the imaging surface ($0.2 \pm 0.02^\circ\text{C}$).

To simulate severe hypothermia (SH), left ventricular canine wedge preparations from 6 dogs were cooled from baseline temperature of 36°C to 26°C at a rate of 11°C/h, with measurements every 2°C (SH group). This rate was used to achieve temperatures typically associated with hypothermia-induced VF and to ensure stability of the preparation during the duration of the experiments. The wedges were then rewarmed to baseline temperature of 36°C at the same rate. To simulate TH, wedges from a separate set of animals were cooled from 36°C to 32°C at a rate of 3°C/h ($n=7$) and subsequently rewarmed (TH group). This rate was chosen to simulate current therapeutic hypothermia protocols.^{1,2} A second therapeutic hypothermia group was cooled and rewarmed at faster rate (9°C/h, $n=8$, fast TH group), similar to that of the SH group, to examine the effects of the rate of cooling and rewarming on arrhythmia substrates.

Data Analysis

APD was measured by using an average of 5 epicardial (EPI), midmyocardial (M), and endocardial (ENDO) cells, respectively, at each temperature. Transmural cell types were defined by previously validated anatomic and functional criterion.^{25,28} Epicardial cells were defined as cells 1 to 2 mm from the epicardial surface. ENDO cells were defined as cells 1 to 2 mm from the endocardial surface.²⁵ M cells were defined as the cells with the longest APD that were not EPI or ENDO cells.²⁵ DOR was defined as the difference between the APD of the longest and shortest cell type. T-peak to T-end (Tp-Te) was also measured in all groups as a more global and clinically applicable measurement of DOR. Tp-Te was measured by the distance of the peak of the T-wave to the end of the T-wave. Conduction velocity (CV) and repolarization time gradients were determined by a previously validated vector analysis technique.²⁸ To assess arrhythmia susceptibility, an identical programmed electric stimulation (PES) protocol was performed at each temperature and in each group.²⁹ During endocardial pacing (S1), up to 2 premature beats were delivered from the epicardial surface (S2 and S3), until failure to capture the preparation or an arrhythmia was induced. Typically, induced arrhythmias self-terminate in the wedge preparation, allowing for serial comparison of arrhythmia susceptibility in any preparation.

Statistical analysis was performed using SPSS Statistics 17.0 (WinWarp, 2008). Pairwise comparisons were used for repeated-measures analysis of variance (ANOVA) for different temperatures in each separate groups. Repeated-measures ANOVA was also used to compare differences between all three hypothermia groups and between the 2 TH groups. When temperature-dependent differences or group differences were found by ANOVA, comparisons were made between specific means using a least-squares-differences post hoc test. Statistical differences referred to in the text and figures are derived from the least-squares-differences post hoc test. Group effects and differences between specific means were considered significant at $P < 0.05$. All mean data are represented with the value and standard error of the mean. Statistical significance in figures is represented by an asterisk or as otherwise stated in the figure legends.

Results

Severe Cooling and Rewarming Promote DOR and Arrhythmogenesis

The effect of hypothermia on APD, DOR, and conduction time during cooling and rewarming in a representative SH experiment are shown in Figure 1. At baseline, DOR is 28 ms. The repolarization gradient is minimal, as the intrinsic difference in APD of the EPI and M cells is similar to the difference in activation time between the cell types during normal conditions (Figure 1, left). On cooling, there is an increase in APD in all cell types as well as a significant

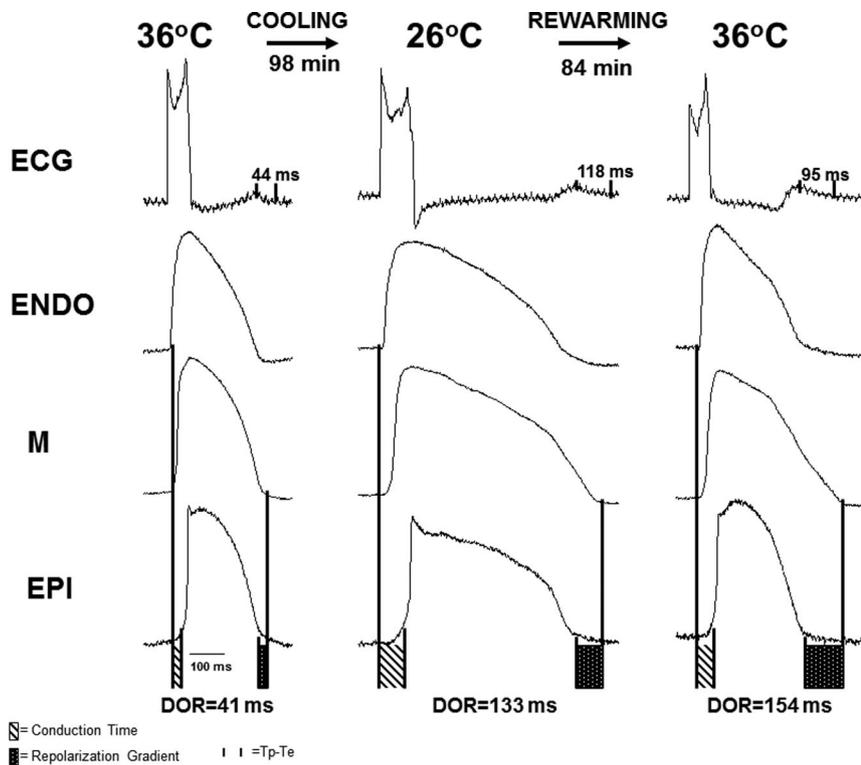


Figure 1. Hypothermia profoundly enhances transmural DOR and slows conduction. Representative action potentials from EPI, M, and ENDO cells during cooling and rewarming are shown. ECG is also shown. Striped shading denotes conduction time; dotted shading denotes repolarization gradient. Tp-Te is shown in red on the ECG. Left, Action potentials at 36°C demonstrate a baseline DOR of 28 ms and CV of 26 cm/s, with a Tp-Te of 44 ms. Middle, When the preparation is cooled to 26°C, marked prolongation of APD, enhancement of DOR, and conduction slowing is observed, with increased Tp-Te. Right, After rewarming to 36°C, conduction time normalizes, but DOR and Tp-Te remains increased, resulting in marked gradients of repolarization.

increase in DOR because of the relatively greater prolongation of M and ENDO cell APD. As expected, conduction time significantly increased during cooling (Figure 1, middle panel). Transmural activation and repolarization are further characterized in Figure 2.

On rewarming, APD of the EPI cells return to near baseline. However, ENDO and M cells remain prolonged and therefore DOR remained elevated. Whereas during cooling, conduction slowing somewhat attenuated repolarization gradients introduced by elevated DOR, during rewarming, conduction time returned to baseline, which further contributed to the significant repolarization gradients introduced during rewarming (Figure 1, right panel, and Figure 2, right panel).

Summary data of DOR and CV for the SH group is shown in Figure 3. Cooling resulted in an increase in both mean APD and DOR. Hypothermia caused a significant increase in mean APD from 224 ± 7 ms to 600 ± 83 ms ($P=0.001$) and an increase in DOR from 26 ± 4 ms to 93 ± 18 ms ($P=0.021$). CV decreased during cooling 35 ± 5 cm/s to 22 ± 5 cm/s ($P=0.010$). The average DOR increased with each increment of cooling. On rewarming, mean APD decreased to 252 ± 18 ms. Importantly, CV returned to baseline (35 ± 2 cm/s), whereas DOR remained significantly prolonged compared with baseline (78 ± 29 , $P=0.021$). Tp-Te measurements closely correlated with DOR. Tp-Te increased from 44 ± 2 ms at baseline to 120 ± 7 ms at 26°C, and, on rewarming to 36°C, remained elevated when compared with baseline (86 ± 4 ms, $P=0.035$).

Figure 4 demonstrates a representative arrhythmia induced by PES in the severe hypothermia group. ECG and action potentials from ENDO, M, and EPI cells during baseline endocardial pacing (S1) are followed by 2 epicardial stimu-

lated beats (S2 and S3), shown in the top panel (3A), whereas transmural conduction and repolarization time maps for each beat are shown below (panel 3B). On the second epicardial premature beat (S3), block occurs (thick red line, S3 activation map, panel 3B) in the subendocardial region precisely at the location of the steepest gradients of repolarization (crowding of isochrones on S2 beat, repolarization map, lower panel, 3B). Block is followed by initiation of polymorphic ventricular tachycardia (VT). These data demonstrate that during severe hypothermia, enhanced DOR, caused by heterogeneous prolongation of myocardial cells, produces a substrate for conduction block and reentrant arrhythmias.

The Table (left column) summarizes the arrhythmia susceptibility for the severe hypothermia group. No spontaneous or induced arrhythmias occurred on cooling to 34°C. On further cooling, conduction block with reentry was induced in 6 of 6 preparations. In 1 preparation, VT was induced and another preparation developed persistent spontaneous VF. Interestingly, the incidence of VT/VF was greater during rewarming. Spontaneous VF was observed in 2 preparations, and reentrant polymorphic ventricular tachycardia was induced in 2 preparations. These data suggest that enhanced transmural DOR induced during cooling and maintained during rewarming is a mechanism of hypothermia-induced arrhythmogenesis, with rewarming being particularly arrhythmogenic.

In Therapeutic Cooling and Rewarming, Hypothermia-Induced DOR and Arrhythmogenesis Is Attenuated

A second group of preparations ($n=7$) was cooled to 32°C and rewarmed at a slower rate to model clinical TH. In the TH

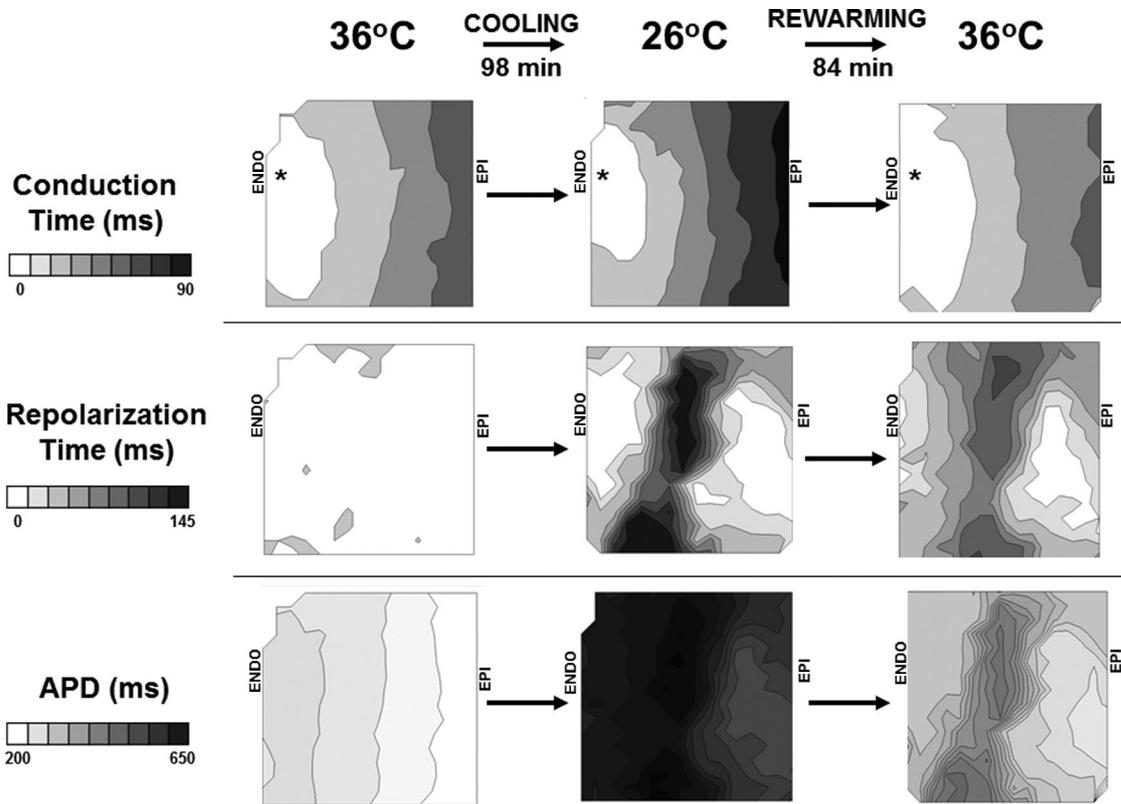


Figure 2. Rewarming from severe hypothermia promotes persistent enhancement of transmural dispersion of APD and repolarization gradients, whereas conduction time returns to baseline. Activation maps of the transmurals surface of the canine wedge preparation for conduction time, repolarization time, and APD at baseline (36°C), cooling to 26°C, and returning to baseline (36°C). Top, Cooling resulted in an increase in conduction time with return to baseline on rewarming. Middle, Repolarization time gradients are enhanced during cooling and on rewarming. Bottom, APD markedly and heterogeneously prolonged during cooling and returned near baseline on rewarming, but, importantly, significant heterogeneities of APD remain on rewarming.

group, cooling mildly increased mean APD and DOR. Mean APD increased from 211 ± 8 ms to 310 ± 12 ms ($P < 0.001$) and returned toward baseline on rewarming (249 ± 9 ms, $P = 0.007$ compared with baseline). CV decreased from 30 ± 3 cm/s to 25 ± 5 cm/s ($P = 0.026$) on cooling to 32°C and returned to baseline on rewarming (27 ± 6 cm/s, $P = \text{NS}$ compared with baseline). DOR increased from 31 ± 6 ms to 50 ± 9 ms ($P = 0.012$), similar to what was seen in the SH group at the same degree of hypothermia. However, a

persistent prolongation of DOR was not observed during rewarming in the TH group (Figure 5, open bars), as DOR returned to baseline (39 ± 7 ms, $P = \text{NS}$ compared with baseline during rewarming). Tp-Te measurements again closely correlated with DOR, increasing from 50 ± 5 ms at baseline to 65 ± 10 ms at 32°C ($P = 0.49$) and subsequently returned to baseline (43 ± 2 ms).

The Table (right column) summarizes the arrhythmias seen in the TH group. Compared with the SH group, there

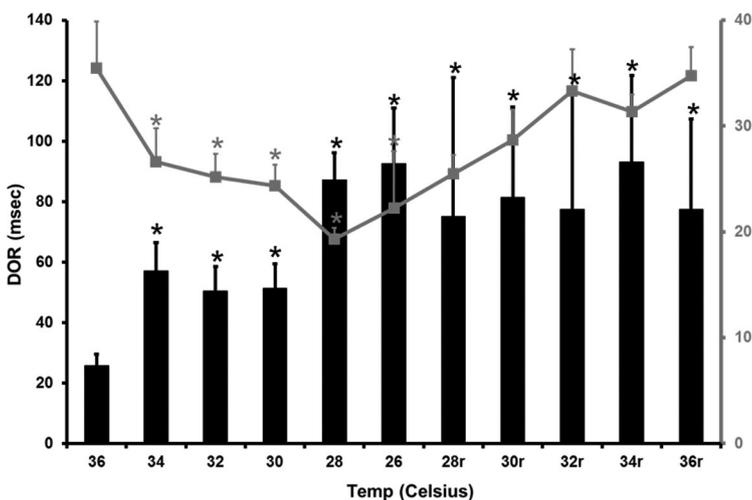


Figure 3. Hypothermia enhances DOR and slows conduction, but DOR remains increased after rewarming. Summary data of DOR and CV is shown for the left ventricle (n=6) during cooling and subsequent rewarming (r). Importantly, although CV returns to baseline during rewarming, DOR remains increased. Compared with baseline, all DOR statistically significant (denoted by asterisk). For CV, statistical significance from baseline (denoted by asterisk).

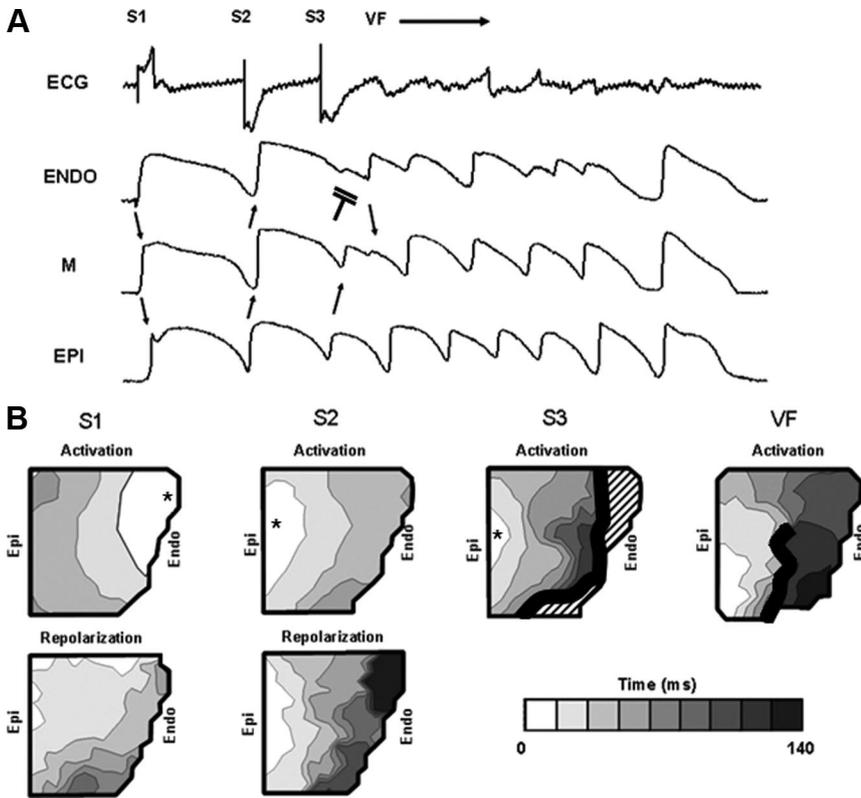


Figure 4. Arrhythmia induced during hypothermia (32°C) using PES. A, ECG and action potentials shown during baseline endocardial pacing (S1) are followed by 2 epicardial stimulated beats (S2 and S3), which initiate conduction block followed by VT. B, Activation and repolarization maps for each beat in A are shown. Pacing site is noted by an asterisk. On the second epicardial premature beat (S3), block occurs (thick red line) in the M-cell region, precisely at the location of the steepest gradients of repolarization (crowding of isochrones on S2 beat, lower panel). Block is followed by initiation of VT. Enhanced DOR, predominantly caused by marked hypothermia-induced prolongation of M and ENDO cells, produces a substrate for conduction block and reentrant arrhythmias.

was a decrease in conduction block, reentry, or VT on cooling (2/7 versus 6/6) and VT during rewarming (1/7 versus 4/5).

Increasing Rate of Cooling and Rewarming Does Not Increase DOR in Therapeutic Hypothermia

To independently assess the effects of rate of temperature change, a second group of TH experiments were performed using increased rates of cooling and rewarming (9°C/h, n=8). During cooling, similar to the slow TH group, APD increased from 244±10 ms to 356±19 ms (P=0.001) and CV decreased from 32±2 cm/s to 25±2 cm/s (P=0.016). On

rewarming to 36°C, mean APD shortened mildly from baseline (226±12 ms, P=NS). CV also returned to baseline (32±2 cm/s, P=NS compared with baseline). DOR did not vary significantly on cooling and rewarming. Tp-Te significantly increased from 41±2 ms at baseline to 60±7 (P=0.018) at 32°C and subsequently returned to baseline.

Figure 5 shows summary data for both TH groups (labeled slow and fast) compared with the SH group at similar temperatures. Increasing the rate of cooling did not significantly alter mean APD, DOR, or CV when compared with the slow TH group. When compared with the 2 TH groups, DOR remained increased in the SH group on rewarming at 34°C and 36°C (SH versus TH slow at 34 and 36 P=0.000, 0.036,

Table. Arrhythmia Susceptibility for the Severe Hypothermia and Therapeutic Hypothermia Groups

Experiment	Severe Hypothermia		Therapeutic Hypothermia	
	Cooling	Warming	Cooling	Warming
1	32°C to 26°C Inducible block and reentry	34°C Inducible block and reentry	No inducible arrhythmia	34°C Inducible block and reentry
2	30°C Inducible block and reentry	32°C Inducible VT	No inducible arrhythmia	No inducible arrhythmia
3	32°C Inducible block and reentry 26°C Spontaneous VF	Not rewarmed	No inducible arrhythmia	32°C Inducible block and reentry 34°C Inducible VT
4	30°C Spontaneous VT	28°C Spontaneous VT 30°C spontaneous VF	32°C Inducible VT	No inducible arrhythmia
5	32°C Inducible block and reentry	28°C Inducible block and reentry 32°C Inducible VT	No inducible arrhythmia	No inducible arrhythmia
6	26°C Inducible block and reentry	26°C Spontaneous VF	No inducible arrhythmia	No inducible arrhythmia
7			32°C Spontaneous VT	34°C Block and reentry

Inducible indicates induced with PES; spontaneous, occurred during observation without PES. Temperature noted is where arrhythmia was first induced or observed during cooling or rewarming protocol. Block and reentry indicates conduction block and single reentrant beat mapped during PES. All VT was nonsustained polymorphic unless otherwise noted. All arrhythmias resolved spontaneously.

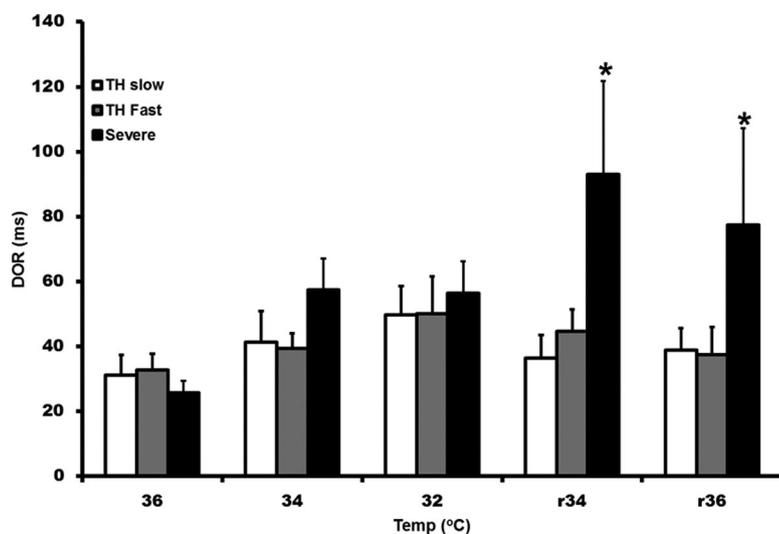


Figure 5. Increasing the temperature rate of change does not enhance DOR. Summary data of DOR is shown for left ventricle during cooling in the slow TH group (3°C/h, n=7) the fast TH group (9°C/h, n=8), and the SH group for comparison with subsequent rewarming (r). Statistical differences between the severe compared with the 2 TH groups are noted (* $P < 0.05$). No significant difference is seen between the 2 TH groups.

respectively; SH versus TH fast at 34 and 36 $P = 0.002$, 0.027 , respectively). Importantly, arrhythmia susceptibility was not further enhanced in the fast TH group. These data suggest that it is the depth of cooling rather than the rate of cooling that is most important in determining arrhythmogenesis in hypothermia.

Discussion

Our data in a model of severe hypothermia demonstrated that enhanced transmural DOR is a mechanism for arrhythmogenesis in hypothermia. Optical mapping revealed that hypothermia heterogeneously prolonged APD and that conduction block and reentry (initiating V_T) occurred precisely where hypothermia-induced APD gradients were greatest. Arrhythmia susceptibility increased as the preparations were cooled and correlated with increased DOR. As the preparations were rewarmed, CV returned to baseline while DOR remained increased (due to more rapid normalization of APD in EPI myocytes than M or ENDO), further enhancing both repolarization gradients and arrhythmia susceptibility. Cooling and rewarming to temperatures typically used clinically in TH was not arrhythmogenic. Although CV slowed and APD was increased during TH, there was no significant increase in DOR observed, suggesting that it is only when hypothermia enhances DOR is arrhythmia susceptibility increased. Only 1 sustained arrhythmia was observed during rewarming during TH and, interestingly, occurred in the preparation with the largest hypothermia-induced enhancement of DOR.

It was interesting that, although the depth of hypothermia was associated with arrhythmias, more arrhythmias were seen during rewarming in the SH group. Steep gradients of repolarization are a substrate for conduction block and reentrant excitation. The profound conduction slowing during induction of hypothermia we observed may attenuate arrhythmia susceptibility because it attenuates hypothermia-induced repolarization gradients (Figure 1). During rewarming, conduction velocity normalized faster than DOR, which contributed to enhanced repolarization gradients and therefore created a persistent substrate for arrhythmogenesis.

This phenomenon was not observed in the TH group. Importantly, these data, and specifically the attenuated DOR and repolarization gradients observed at temperatures targeted during TH, may provide a mechanistic explanation of the low clinical incidence of arrhythmias in patients treated with TH.

It is likely that several temperature-dependent sarcolemmal ion channels play a role in the heterogeneous APD prolongation and arrhythmogenesis observed during severe hypothermia compared with TH, potentially including I_{Kr} and I_{Ks} as well as I_{Na} , I_{to} , and $I_{Ca,L}$.^{11–19} Additional mechanisms by which hypothermia might also enhance DOR include hypothermia-induced intracellular uncoupling, which might enhance intrinsic transmural repolarization heterogeneities, as well as contribute to the conduction slowing observed secondary to hypothermia.^{30,31} Further studies will be required to investigate the cellular and subcellular mechanisms responsible for hypothermia-induced enhancement of DOR.

Limitations

These experiments have several limitations. First, electrophysiological effects of hypothermia were evaluated only in the canine wedge preparation and therefore in a 2-dimensional area of a small portion of the left ventricle. Although these data suggest that transmural heterogeneities may be responsible for hypothermia-induced ventricular arrhythmias, as we did not examine heterogeneities in the intact heart in our experiments, we cannot exclude that other hypothermia induced repolarization heterogeneities may be important in hypothermia-related arrhythmogenesis. We cannot account for changes in interventricular conduction or repolarization or other heterogeneities (such as apex to base heterogeneities), which could potentially play a role in arrhythmia susceptibility. We did, however, examine the effects of hypothermia in canine wedges taken from the right ventricle which demonstrated similar temperature-dependent effects on APD as was observed in left ventricle (data not shown), suggesting that interventricular heterogeneities in response to hypothermia may not be as important.

Based on the stability of the preparation determined from time control experiments, our study protocol was limited to approximately 4 hours, thus limiting the rates of cooling and rewarming that could be studied. In the accidental hypothermia protocol, the average cooling and rewarming was approximately 11°C/h. This probably is a faster cooling rate than typically occurs in most cases of accidental hypothermia (although this may be observed in cold water submersion). Unfortunately, we were unable to study slower rates, and these rates were chosen to ensure complete stability of preparation and arrhythmia substrates throughout the protocol. The TH model was closer to likely clinical conditions (typically 1 to 2°C/h)^{1,2} with cooling and rewarming at 3°C/h. Our study was limited to cooling to a low temperature of 26°C. We found that further cooling to lower temperatures prevented maintaining viable preparations for rewarming, as in preliminary studies cooling further caused refractory VF in these preparations.

Conclusion

Hypothermia heterogeneously affects repolarization of ventricular cells, which amplifies DOR and represents a mechanism for arrhythmogenesis. Hypothermia-induced changes in DOR and resultant arrhythmia susceptibility is dependent on the severity of hypothermia during both cooling and rewarming. Importantly, there is an increased risk of arrhythmogenesis during rewarming during severe hypothermia, secondary to a persistent elevation in DOR. In our studies, cooling and rewarming as in TH resulted in a mild increase in DOR and an attenuation of arrhythmogenesis. This observation may explain why therapeutic hypothermia is not associated with significant arrhythmogenic risk, because current protocols induce only mild hypothermia slowly and rewarm gradually over 24 hours. The increased DOR and incidence of arrhythmias observed during rewarming from severe hypothermia suggests that patients are at highest risk for arrhythmogenesis during rewarming from severe hypothermia. As Tp-Te correlated closely with arrhythmogenic DOR during hypothermia and rewarming, these data suggest that it may provide a clinically useful metric for evaluation of arrhythmogenic substrates in hypothermia.

Sources of Funding

This study was supported by a Departmental MetroHealth Foundation Grant (Dr Piktel), an Emergency Medicine Foundation Career Development Grant (Dr Wilson), and by National Institutes of Health Grant RO1-HL54807 (Dr Rosenbaum).

Disclosures

None.

References

- Hypothermia After Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve outcome after cardiac arrest. *N Engl J Med.* 2002; 346:549–556.
- Bernard SA, Gray TW, Buist M, Jones BM, Silvester W, Gutteridge G, Smith K. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med.* 2002;346:557–563.
- Nolan JP, Morley PT, Vanden Hoek TL, Hickey RW. Therapeutic hypothermia after cardiac arrest: an advisory statement by the Advanced Life Support Task force of the International Liaison Committee on Resuscitation. *Circulation.* 2003;108:118–121.
- Murphy T, Zumwalt R, Fallico F, Sanchez C, Belson M, Rubin C, Azziz-Baumgartner E. Hypothermia-related deaths—United States, 1999–2002 and 2005. *MMWR.* 2006;55:282–284.
- Hypothermia related deaths. *MMWR.* 2006;55:282–284.
- Hauty MG, Esrig BC, Hill JG, Long WB. Prognostic factors in severe accidental hypothermia: experience from the Mt Hood tragedy *J Trauma.* 1987;27:1107–1112.
- 2005 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation.* 2005; 112(24 suppl):IV-84–IV-88.
- Vassal T, Benoit-Gonin B, Carrat F, Guidet B, Maury E, Offenstadt G. Severe accidental hypothermia treated in an ICU, prognosis and outcome. *Chest.* 2001;120:1998–2003.
- Yan GX, Antzelevitch CA. Cellular basis for the electrocardiographic J wave. *Circulation.* 1996;93:372–379.
- Durakovic Z, Misigoj-Durakovic M, Corovic N, Curbillo-Turek M, Turek S. The corrected Q-T interval in the elderly with urban hypothermia. *Coll Antropol.* 1999;23:683–690.
- Mortensen E, Berntsen R, Tveita T, Lathrop DA, Refsum H. Changes in ventricular fibrillation threshold during acute hypothermia: a model for future studies. *J Basic Clin Physiol Pharmacol.* 1993;4: 313–319.
- Gambassi G, Cerbai E, Pahor M, Capogrossi MC, Carboni P, Mugelli A. Temperature modulates calcium homeostasis and ventricular arrhythmias in myocardial preparations. *Cardiovasc Res.* 1994;28: 391–399.
- Liu B, Arlock P, Wohlfart B, Johansson BW. Temperature effects on the Na and Ca currents in rat and hedgehog ventricular muscle. *Cryobiology.* 1991;28:96–104.
- Suleiman MS, Chapman RA. Effect of temperature on the rise of intracellular sodium caused by calcium depletion in ferret ventricular muscle and the mechanism of the alleviation of the calcium paradox by hypothermia. *Circ Res.* 1990;67:1238–1246.
- Marshall C, Elias C, Xue X, Dinh Le H, Omelchenko A, Hryshko LV, Tibbitts GF. Determinants of cardiac Na⁺/Ca²⁺ exchanger temperature dependence: NH₂-terminal transmembrane segments. *Am J Physiol Cell Physiol.* 2002;283:512–520.
- Melinikov AL, Lathrop DA, Helgesen KG. Diazepam-induced Ca²⁺ channel blockade reduces hypothermia-induced electromechanical changes in isolated guinea ventricular muscle. *Eur J Anesth.* 1998;15: 96–102.
- Sprung J, Laszlo A, Turner LA, Kampine JP, Bosnjak ZJ. Effects of hypothermia, potassium, and verapamil on the action potential characteristics of canine cardiac Purkinje fibers. *Anesthesiology.* 1995;2: 713–722.
- Sprung J, Stowe DF, Kampine JP, Bosnjak ZJ. Hypothermia modifies anesthetic effect on contractile force and Ca²⁺ transients in cardiac Purkinje fibers. *Am J Physiol.* 1994;36:725–733.
- Bjørnstad H, Jensen D, Mortensen E. Diltiazem does not increase ventricular fibrillation threshold during hypothermia. *Acta Anesthesiol Scand.* 1995;39:659–665.
- Salama G, Kanai AJ, Huang D, Efimov RD, Girouard SD, Rosenbaum DS. Hypoxia and hypothermia enhance spatial heterogeneities of repolarization in guinea pig hearts: analysis of spatial autocorrelation of optically recorded action potential durations. *J Cardiovasc Electrophysiol.* 1998;9: 164–183.
- Kuo CS, Reddy CP, Munkata K, Surawicz B. Mechanism of ventricular arrhythmias caused by increased dispersion of repolarization. *Eur Heart J.* 1985;6(Suppl D):63–70.
- Akar FG, Yan GX, Antzelevitch C, Rosenbaum DS. Unique topographical distribution of M cells underlies reentrant mechanism of torsade de pointes in the long-QT syndrome. *Circulation.* 2002;105: 1247–1253.
- Akar FG, Rosenbaum DS. Transmural electrophysiological heterogeneities underlying arrhythmogenesis in heart failure. *Circ Res.* 2003;93: 638–645.

24. Morita H, Zipes D, Morita S, Wulfs J. Temperature modulation of ventricular arrhythmogenicity in a canine tissue model of Brugada syndrome. *Heart Rhythm*. 2007;4:2:188–189.
25. Girouard SD, Laurita KR, Rosenbaum DS. Unique properties of cardiac action potentials recorded with voltage-sensitive dyes. *J Cardiovasc Electrophysiol*. 1996;7:1024–1038.
26. Rosenbaum DS, Akar FG. The electrophysiologic substrate for reentry: unique insights from high-resolution optical mapping with voltage-sensitive dyes. In: Cabo C, Rosenbaum DS, eds. *Quantitative Cardiac Electrophysiology*. New York, NY: Marcel Dekker Inc, 2002:555–582.
27. Akar FG, Laurita KR, Rosenbaum DS. Cellular basis for dispersion of repolarization underlying reentrant arrhythmias. *J Electrocardiol*. 2000; 33(Suppl):23–31.
28. Pajouh M, Wilson LD, Poelzing S, Johnson N, Rosenbaum DS. I_{Ks} blockade reduces dispersion of repolarization in heart failure. *Heart Rhythm*. 2006;2:7:731–738.
29. Fouts K, Fernandes B, Mal N, Liu J, Laurita K. Electrophysiological consequences of skeletal myoblast transplantation in normal infarcted canine myocardium. *Heart Rhythm*. 2006;3:452–461.
30. Poelzing S, Roth BJ, Rosenbaum DS. Optical measurements reveal nature of intercellular coupling across ventricular wall. *Am J Physiol Heart Circ Physiol*. 2005;289:H1428–H1435.
31. Fedorov VV, Glukhov AV, Sudharshan S, Egorov Y, Rosenshtraukh LV, Efimov IR. Electrophysiological mechanisms of antiarrhythmic protection during hypothermia in winter hibernating versus nonhibernating mammals. *Heart Rhythm* 2008;5:11:1587–1596.

CLINICAL PERSPECTIVE

With increasing use of therapeutic hypothermia (TH) in the care of patients resuscitated from cardiac arrest, it is critically important to understand the electrophysiologic effects of hypothermia on cardiac myocytes and arrhythmia substrates. In the present study, we tested the hypothesis that hypothermia-enhanced transmural dispersion of repolarization (DOR) is a mechanism of arrhythmogenesis in hypothermia using optical mapping of the transmural wall of the canine left ventricle. The electrophysiologic effects of severe hypothermia (cooling to 26°C) was compared with therapeutic hypothermia (TH, cooling to 32°C), and rate of cooling was also investigated (9°C/h versus 3°C/h). Severe hypothermia significantly increased DOR, promoting conduction block, reentrant excitation, and arrhythmogenesis. During rewarming, DOR remained elevated and arrhythmia susceptibility remained increased. During TH, the increase in DOR was mild and arrhythmia susceptibility was decreased, and the rate of cooling and rewarming did not alter arrhythmia susceptibility. Because DOR and resultant arrhythmia susceptibility was directly dependent on the severity of hypothermia during both cooling and rewarming, this may explain why TH is not associated with significant arrhythmogenic risk. Our data suggest that clinical protocols used to cool and rewarm patients should maintain hypothermia in the mild range ($\geq 32^\circ\text{C}$) and suggest that patients are at highest risk for arrhythmogenesis during rewarming. In addition, because T-peak to T-end of the ECG closely correlated with DOR and arrhythmogenesis during hypothermia, this may represent clinical marker for arrhythmia risk during both therapeutic hypothermia and rewarming for severe hypothermia.

Enhanced Dispersion of Repolarization Explains Increased Arrhythmogenesis in Severe Versus Therapeutic Hypothermia

Joseph S. Piktel, Darwin Jeyaraj, Tamer H. Said, David S. Rosenbaum and Lance D. Wilson

Circ Arrhythm Electrophysiol. 2011;4:79-86; originally published online December 16, 2010;
doi: 10.1161/CIRCEP.110.958355

Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2010 American Heart Association, Inc. All rights reserved.

Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circep.ahajournals.org/content/4/1/79>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Arrhythmia and Electrophysiology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Circulation: Arrhythmia and Electrophysiology* is online at:
<http://circep.ahajournals.org/subscriptions/>