

# Effects of Local Epicardial Cooling/Warming on the Complexity of the Ventricular Fibrillatory Pattern

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## Abstract

*In ten Langendorff-perfused rabbit hearts localized cooling/warming were induced by a specific device in a ventricular epicardium area (modified zone, MZ) during ventricular fibrillation (VF). Epicardial electrical activity was mapped in MZ and another area of the same ventricle (NMZ), during basal temperature (37 °C), MZ-hypothermia (32 °C, 27 °C, 22 °C) and MZ-hyperthermia (42 °C). We analyzed: 1) the mean fibrillatory interval (VV) at each temperature step, and 2) the complexity of the activation maps (I, II and III classification, in increasing order) at basal temperature, hypothermia-22 °C and hyperthermia. In MZ, VF was decelerated by hypothermia (VV: 65 ± 16 vs 52 ± 10 ms,  $p < 0.05$ ) and accelerated by hyperthermia (VV: 44 ± 7 vs 52 ± 7 ms,  $p < 0.05$ ), in a reversible manner. VV changes with temperature in MZ fitted to a lineal model ( $r = -0.52$ ,  $p < 0.0001$ ). In NMZ, no significant differences were observed. Epicardial hypothermia induced a significant variation in the complexity of the VF activation maps in MZ respect NMZ, with type III increments and type II decrements (MZ-22 °C: I = 7%, II = 31%, III = 62%; NMZ-22 °C: I = 7%, II = 47%, III = 46%,  $p < 0.01$ ). We conclude that local myocardial warming accelerates VF, and local myocardial cooling decelerates VF and increases the complexity of the VF activation pattern.*

## 1. Introduction

The ventricular fibrillation (VF) is a lethal arrhythmia and when this happens it causes the heart contraction lost, the annulment of the cardiac beat and the sudden death of the patient and this is studied from different points of view [1,2]. The progress made on the knowledge of the basic mechanisms which determinates its beginning, its perpetuation or its cease altogether, are necessary to make a progress on the prevention of the VF as well as to work on the efficiency of the procedures used to interrupt it adequately. A great deal of progress has been made

thanks to the investigation on experimental models based on isolated perfused animal hearts, being these a great methodological support highly used nowadays on the arrhythmia studies [3,4].

On many of these studies it is used the modulating effects which some agents have over the myocardial electrophysiology, being the temperature one of these agents. The temperature affects the electrophysiology of the cardiac cells [5,6]. The hypothermia as much as the hyperthermia modify parameters like the refractoriness and the conduction velocity of the cardiac tissue [7,8], and can be related to a great or minor ease for the establishment or cease of ventricular arrhythmias [9-11]. On the same way, the analysis of the dynamics of conduction during the thermal modifications, can bring forward important information on the mechanisms of the VF or on the effects of this variable. The frequency or the complexity of the activation characterizes this dynamics. The frequency of activation is associated to the mean fibrillatory interval or the dominant frequency obtained out of the electrograms [12]. Regarding the complexity, a possibility to determinate this is based on the identification of the conduction patterns on the myocardial activation maps and its later classification by levels [13]. Using this methodology, it was proved that the acute and global myocardial cooling [14] induced to the cease of the VF and this was preceded by a significant decrease of the complexity of the arrhythmia. Nevertheless, systematic studies have not been done to analyse the effect that the localized myocardial temperature modifications have over the complexity of the VF, although it has to be point out that studies have been done on the variations on the dominant frequency of the activation [15].

## 2. Method

### 2.1. Experimental preparation

This study complies with the Guide for the Care and

Use of Laboratory Animals published by the US National Institutes of Health [DHHS Publication no. (NIH) 85-23, revised 1996], and with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European Community, 24 November 1986). Ten New Zealand rabbits (mean weight =  $2.5 \pm 0.3$  kg) were used (methodological details in [15]), an epicardial mapping electrode with an integrated thermoelectric device (128 unipolar electrodes, 1 mm-pitch) [16,17] was applied to the anterior wall of the left ventricle (MZ). For control, a conventional stainless-steel electrode array (103 unipolar electrodes, 1 mm-pitch) was positioned in the epicardial surface of the posterolateral wall of the same ventricle (NMZ). A K-type thermocouple was manually placed in an area of the ventricular epicardium away from the modified area, specifically in the posterior wall of the left ventricle, very close to the junction with the right ventricle. An external bipolar electrode was placed in the part of the epicardium situated between the areas covered by both electrodes, with a view to inducing ventricular fibrillation via stimulation using increasing frequencies (Figure 1). Recordings were obtained with a cardiac electrical activity mapping system (MAPTECH, Waalre, The Netherlands). The reference electrode was a 4mm×6mm silver plaque located over the cannulated aorta. All signals were amplified with a gain of 100–300, filtered (bandwidth = 1–400 Hz), multiplexed and digitized (resolution = 12 bit). The sampling rate per channel was 1000 Hz. Temperatures from thermocouples were recorded by means of Fluke® digital thermometers (Fluke Co., Everett, WA, USA).

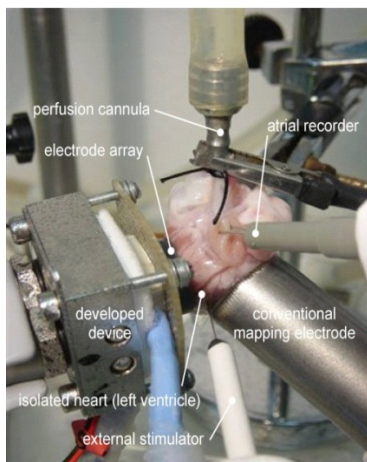


Figure 1. Isolated perfused heart and the different elements during an experiment.

## 2.2. Experimental protocol

With the electrodes placed, VF was induced by pacing, to register the fibrillatory signal at different temperatures. Five minutes after the VF onset, the temperature in MZ

was reduced in 5 °C steps by regulating the electric current of the thermoelectric cooler. After reaching the lowest step (22 °C), the temperature was incremented in the same way to 42 °C (by inverting polarity at the terminals of the Peltier device), followed by a return to the baseline. At each step (in this order: 37 °C, 32 °C, 27 °C, 22 °C, 37 °C, 42 °C and 37 °C), the epicardial temperature was stabilized ( $\pm 0.2$  °C) during at least 2 minutes before recording.

## 2.3. Analysis of VF

*Time-domain analysis of VF.* Activation times in each channel were automatically determined [18]. The fibrillatory intervals and the mean of the consecutive intervals (VV) during three 8-s time windows were determined for both, MZ and NMZ.

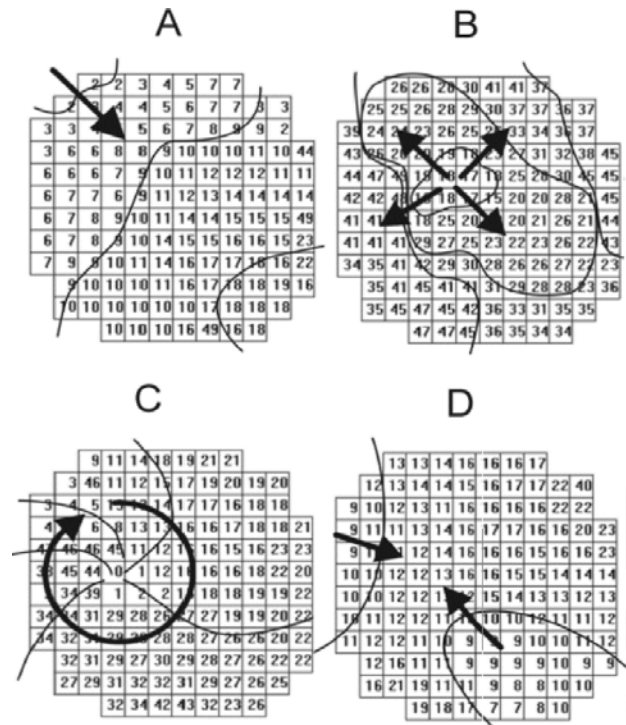


Figure 2. Different types of activation patterns obtained in MZ. Single wavefront (A), breakthrough pattern (B), reentrant activation (C) and wavefront collision (D). Isochrones drawn. Arrows indicating the direction of activation.

*Analysis of epicardial activation maps during VF.* Activation maps during VF were constructed every 100 ms in the 2-s time windows, in both zones. Isochrones were drawn and, in each map, the wavefronts, conduction block lines, reentrant activations, and breakthrough patterns were analyzed as in previous studies [14]. Each map was classified into three categories [13] based in its complexity: type I, single broad wavefronts without conduction block lines or areas of slow conduction; type II, two wavefronts or one wavefront with areas of conduction block or slow conduction; and type III, three

wavefronts associated with areas of slow conduction and conduction block.

On figure 2 it is shown examples of activation patterns. They explain the times of ventricular activation on each channel and the correspondent isochrones. According to the criteria used, the correspondence would be: A-I; B-I, C-II and D-II.

*Statistical Analysis.*- Data are presented as the mean  $\pm$  standard deviation. The repeated-measures ANOVA procedure was used to perform multiple comparisons. The paired Student t-test was used to compare individual pairs of data. The analysis of dependence between variables was done using the Chi-squared test for contingency tables. Differences were considered to be statistically significant at  $p < 0.05$ . The linear regressions between pairs of variables were made using the least-square method. All data summaries and statistical analyses were performed using Excel 2007 (Microsoft, Redmond, Washington) and the SPSS 11.0 statistical package (SPSS Inc, Chicago, IL).

Table 1. Temperatures ( $^{\circ}\text{C}$ ) during VF in MZ (TM) and NMZ (TN), and VV intervals (ms) in MZ (VVM) and NMZ (VVN). Values are mean  $\pm$  standard deviation (n=10), \* $p < 0.05$  vs baseline.

TM	TN	VVM	VVN
37.3 $\pm$ 0.4	37.5 $\pm$ 1.3	52 $\pm$ 10	52 $\pm$ 9
31.9 $\pm$ 0.2	37.9 $\pm$ 0.3	55 $\pm$ 13	51 $\pm$ 8
27.0 $\pm$ 0.1	37.6 $\pm$ 0.8	60 $\pm$ 13*	51 $\pm$ 10
22.1 $\pm$ 0.2	37.4 $\pm$ 0.9	65 $\pm$ 16*	53 $\pm$ 9
37.2 $\pm$ 0.4	37.7 $\pm$ 0.7	52 $\pm$ 7	52 $\pm$ 7
42.0 $\pm$ 0.5	37.8 $\pm$ 0.6	44 $\pm$ 7*	51 $\pm$ 6
37.2 $\pm$ 0.4	37.7 $\pm$ 0.6	48 $\pm$ 8	51 $\pm$ 6

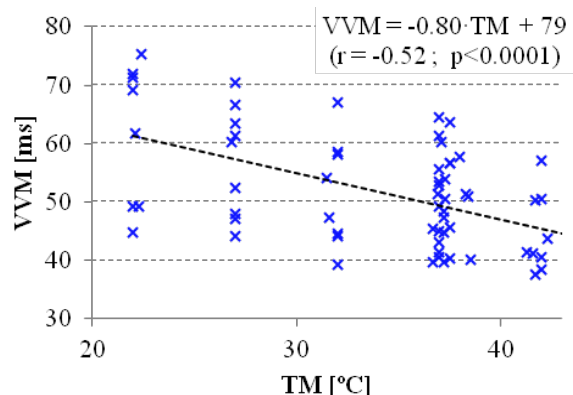


Figure 3. Relationship between fibrillatory interval (VVM) and temperature (TM) in MZ, from determinations at different steps. Dashed line represents the least-square linear regression (61 points, n=10).

### 3. Results

The temperature and the mean fibrillatory interval data

obtained on both zones for each step of temperature, is shown in Table 1. In MZ, for baseline, thermally induced changes have led to changes in the VV. Thermally induced changes are reversible. Only at 32  $^{\circ}\text{C}$  variations do not reach statistical significance, although there are variations on the overall of the data. Figure 3 shows the scatter plot VVM-TM and the result of the lineal regression, which offers a moderate correlation between both. Hypothermia prolongs the VV and hyperthermia has an opposite effect.

Table 2. Number of activation maps according to their complexity (I, II and III, in order of complexity) observed in MZ and NMZ at baseline (37 $^{\circ}\text{C}$ ), local cooling (22 $^{\circ}\text{C}$ ) and warming (42 $^{\circ}\text{C}$ ) during VF (n=10).

TM Step	Zone	I	II	III
37	MZ	10	87	103
	NMZ	17	78	105
22	MZ	13	64	123
	NMZ	14	94	92
42	MZ	20	80	100
	NMZ	17	93	90

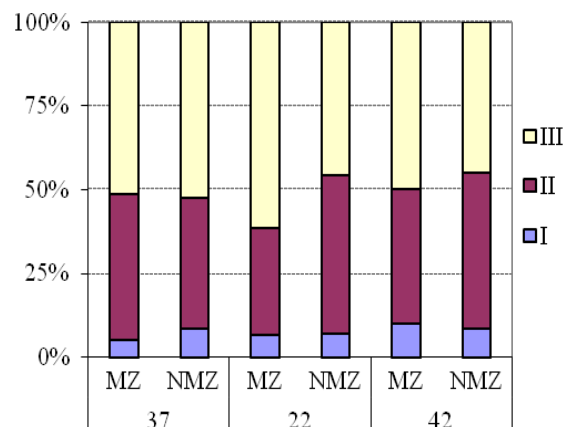


Figure 4. Percentages of types of activation maps according to table 2.

After analyzing the dependence between variables, it has been found that: i) in MZ there is complexity-temperature relationship ( $p < 0.05$ ), and ii) during hypothermia there is complexity-zone relationship ( $p < 0.01$ ). As shown in Table 2 and Figure 4, in MZ, there is an increase of the type III maps and a reduction of the type II maps, during hypothermia. In addition, during cooling, the percentage of type III maps in MZ is higher than NMZ, whereas type II is lower.

In previous studies [14], global and acute hypothermia reduced the complexity of the VF until reaching reversion. Our results, contrary to those, could be

explained because the thermal actions made have reached limited depth [16]. The induced heterogeneity between the epicardial surface (clearly affected) and the deepest layers, would ease an increase on the complexity of the activation.

#### 4. Conclusions

The local temperature changes have induced reversible changes in fibrillatory interval length: heating and cooling accelerates and decelerates the VF respectively, both parameters being inversely correlated. These changes have also affected the complexity of activation during VF, which has been increased in hypothermic conditions.

#### Acknowledgements

This work was supported by The Spanish Science Ministry, Instituto de Salud Carlos III grants FIS PS09/02417, Generalitat Valenciana (Valencian Government): PROMETEO 2010/093 and VLC Campus of Excellence “Cardiovascular Protection”.

#### References

- [1] Deo R, Albert CM. Epidemiology and genetics of sudden cardiac death. *Circulation*. 2012;125(4):620-37.
- [2] Aronow WS. Implantable cardioverter-defibrillators. *Am J Ther*. 2010;17(6):e208-20.
- [3] Chorro FJ, Such-Belenguer L, López-Merino V. Modelos animales de enfermedad cardiovascular. *Rev Esp Cardiol*. 2009;62(1):69-84
- [4] Jalife J. Ventricular fibrillation: mechanisms of initiation and maintenance. *Annu Rev Physiol*. 2000; 62: 25–50.
- [5] Arnaud C, Joyeux M, Garrel C, Godin-Ribuot D, Demenge P and Ribuot C. Free-radical production triggered by hyperthermia contributes to heat stress-induced cardioprotection in isolated rat hearts *Br. J. Pharmacol*. 2002;135:1776–82
- [6] Bjornstad H, Tande PM, Lathrop DA and Refsum H. Effects of temperature on cycle length dependent changes and restitution of action potential duration in guinea pig ventricular muscle *Cardiovasc. Res*. 1993;27:946–50.
- [7] Salama G, Kanai AJ, Huang D, Efimov IR, Girouard SD and 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;9:164–83.
- [8] Nygren A, Kondo C, Clark RB and Giles WR. Voltage-sensitive dye mapping in Langendorff-perfused rat hearts *Am. J. Physiol. Heart Circ. Physiol*. 2003; 284:H892–902.
- [9] Boersma L, Zetelaki Z, Brugada J, Allessie M. Polymorphic reentrant ventricular tachycardia in the isolated rabbit heart studied by high-density mapping. *Circulation*. 2002;105(25):3053-61.
- [10] Harada M, Honjo H, Yamazaki M, Nakagawa H, Ishiguro YS, Okuno Y, Ashihara T, Sakuma I, Kamiya K, Kodama I. Moderate hypothermia increases the chance of spiral wave collision in favor of self-termination of ventricular tachycardia/fibrillation. *Am J Physiol Heart Circ Physiol*. 2008;294(4):H1896-905.
- [11] Yamazaki M, Honjo H, Ashihara T, Harada M, Sakuma I, Nakazawa K, Trayanova N, Horie M, Kalifa J, Jalife J, Kamiya K, Kodama I. Regional cooling facilitates termination of spiral-wave reentry through unpinning of rotors in rabbit hearts. *Heart Rhythm*. 2012;9(1):107-14.
- [12] Chorro FJ, Cánoves J, Guerrero J, Mainar L, Sanchis J, Such L, López-Merino V. Alteration of ventricular fibrillation by flecainide, verapamil, and sotalol: an experimental study. *Circulation*. 2000;101(13):1606-15.
- [13] Konings KT, Kirchhof CJ, Smeets JR, Wellens HJ, Penn OC, Allessie MA. High-density mapping of electrically induced atrial fibrillation in humans. *Circulation* 1994;89(4):1665-80.
- [14] Chorro FJ, Guerrero J, Ferrero A, Tormos A, Mainar L, Millet J, Cánoves J, Porres JC, Sanchis J, Lopez-Merino V, Such L. Effects of acute reduction of temperature on ventricular fibrillation activation patterns. *Am J Physiol Heart Circ Physiol*. 2002; 283(6):H2331-40.
- [15] Tormos A, Chorro FJ, Millet J et al. Analyzing the electrophysiological effects of local epicardial temperature in experimental studies with isolated hearts. *Physiol. Meas* 2008 29:711-728.
- [16] Tormos A, Guill A, Millet J, Roses EJ, Trapero I, Such-Miquel L, Chorro FJ. New epicardial mapping electrode with warming/cooling function for experimental electrophysiology studies. *Med Eng Phys* 2011;33:653-9.
- [17] Guill A, Trapero I, Roses E, Millet J, Tormos A, Pelechano F, Such-Miquel LM, Climent AM, Such L, Chorro FJ. QT Dispersion Induced by Local Temperature Variations. *Computers in Cardiology* 2008:697-700.
- [18] Ibáñez-Català X, Guillem M, Climent A, Chorro FJ, Pelechano F, Trapero I, Roses E, Guill A, Tormos A, Millet J. 2D Isochronal correlation method to detect pacing capture during ventricular fibrillation. 4th European Conference of the International Federation for Medical and Biological Engineering 2009:14-17.

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