Epicardial-limited Electrophysiological Heterogeneities do not facilitate Ventricular Arrhythmia Induction. An Experimental Study

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Abstract

The electrophysiological heterogeneities of the myocardium are associated with vulnerability to arrhythmias. This study presents an experimental heterogeneity model based on local epicardial cooling/warming.

The ventricular activation-recovery interval (ARI), conduction velocity (CV) and arrhythmogenic response to electrical pacing were determined. Electrical mapping was carried out on isolated rabbit hearts (n=8), using a specific electrode device for epicardial temperature control.

With respect to baseline, ARI in the modified zone was prolonged (137 ± 22 ms vs 111 ± 13 ms, p<0.05) under maximum hypothermia (22.3 ± 0.6 ºC vs 36.7 ± 0.8 ºC), and was shortened (98 ± 13 ms vs 107 ± 16 ms, p<0.05) under conditions of hyperthermia (41.8 ± 0.3 ºC vs 37.3 ± 0.4 ºC). CV decreased (70 ± 17 cm/s vs 76 ± 17 cm/s, p<0.05) under hypothermia and increased (79 ± 20 cm/s vs 75 ± 21 cm/s, p<0.05) under hyperthermia. There were no changes in the unmodified zone. Repetitive responses were observed in four hearts, with no dependency between the appearance of responses and the induced modifications. Thermally induced dispersion of ARI and CV did not favor the induction of ventricular arrhythmias, probably because only a limited zone of the ventricular epicardium was affected.

1. Introduction

Ventricular arrhythmias are the leading ultimate cause of sudden cardiac death in the industrialized world [1]. Although there have been important advances in our knowledge of the mechanisms giving rise to such arrhythmias and their perpetuation, a number of uncertainties remain [2]. In this context, experimental isolated perfused organ models remain crucial for furthering knowledge in this field [3]. The existence of electrophysiological heterogeneities in cardiac tissue favors the appearance of arrhythmogenic substrates [4]. The dispersion of refractoriness and other properties related to electrical conduction can be reproduced in these models without pharmacological intervention. Given the modulating effect of temperature upon cardiac electrophysiology, myocardial thermal gradients can give rise to heterogeneities with arrhythmogenic potential [5]. The induction of local temperature variations at ventricular epicardial level can be expected to generate heterogeneities facilitating the appearance of arrhythmias. These variations can be induced by using specific customized devices [6]. The present study describes an experimental induced heterogeneity model involving localized thermal variations in a limited region of the ventricular epicardium. Heterogeneity is verified by determining two electrophysiological parameters: the activation-recovery interval (ARI) and conduction velocity (CV). The induction of arrhythmic events in response to programmed electrical pacing is recorded, and their appearance is related to the produced heterogeneity.

2. Methods

Experimental model. The experiments were carried out in abidance with Spanish legislation as specified by Royal Decree 1201/2005. Eight New Zealand rabbits were used (mean weight 2.6 ± 0.2 kg). Following anesthesia with intramuscular ketamine (35 mg/kg) and heparinization, the animals were sacrificed by cervical dislocation. The hearts (mean weight 8.6 ± 0.9 g) were removed and immersed in cold Tyrode solution (4.8 ºC). After isolation, the aorta was connected to a Langendorff system for perfusion of the Tyrode solution at a pressure

of 60 mmHg and a temperature of 37.0 ± 0.5 ºC. The millimolar composition of the perfusion solution was: 130.0 NaCl, 24.2 $NaHCO_3$, 4.7 KCl, 2.2 $CaCl_2$, 1.2 NaH_2PO_4, 0.6 $MgCl_2$ and 12.0 glucose. Oxygenation was performed with a mixture of 95% O_2 and 5% CO_2.

Thermal modifications and electrogram recording. A multiple electrode (Figure 1) was placed in the anterior wall of the left ventricle (modified zone) for epicardial mapping, with the use of an integrated thermoelectric device (128 unipolar electrodes; inter-electrode distance = 1 mm) for temperature control and modification [7]. A conventional multiple electrode (103 unipolar electrodes; inter-electrode distance = 1 mm) was placed at the epicardial surface of the posterolateral wall of the same ventricle (unmodified zone). The temperature of both zones was monitored using type K thermocouples. The first thermocouple was integrated in the recording surface of the modifier device, while the second was positioned in an area of the ventricular epicardium of the posterior wall of the left ventricle distant from the modified zone. The thermocouple temperature values were recorded with Fluke® digital thermometers (Fluke Co.; Everett, Washington, USA). The electrogram recordings were obtained by means of a cardiac electrical activity mapping system (MAPTECH; Waalre, The Netherlands). The reference electrode was positioned over the cannulated aorta. All the signals were amplified (gain 100-300 V/V), filtered (bandwidth 1-400 Hz), multiplexed and digitized (resolution 12 bits). *The sampling frequency per channel was 1 kHz.*

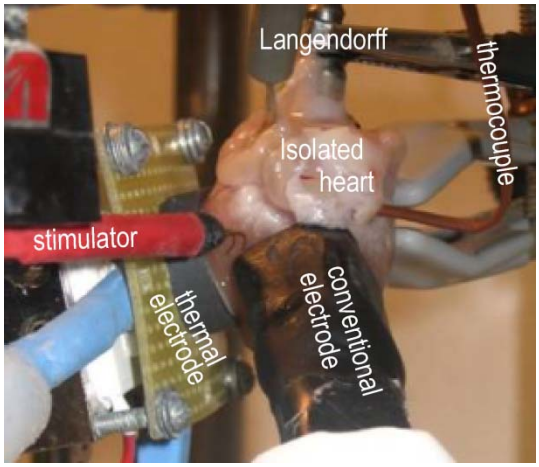

Figure 1. Isolated heart and the different elements used during an experiment.

Epicardial pacing. Use was made of a bipolar electrode (diameter = 0.125 mm; inter-electrode distance = 1 mm) placed between the areas covered by the two electrodes and connected to a GRASS S88 generator equipped with a GRASS SIU5 stimulation isolation unit. The stimuli consisted of a train of rectangular pulses with a duration of 2 ms and a voltage equal to twice the diastolic threshold. The ventricular extrastimulus test with 10 base train impulses was applied, reducing the coupling interval of the additional stimulus in 5-ms steps from base cycle to capture loss. The procedure was repeated with 1, 2 and 3 extrastimuli, in that order. In the case of 2 and 3 extrastimuli, those before the last were set to a value 15 ms greater than the interval in which capture loss occurred in the preceding test. The procedure was repeated for two base cycles: 250 and 150 ms.

Experimental protocol. Fifteen minutes after placing of the electrodes, the temperature of the modified zone was lowered in 5 ºC steps by adjusting the current in the thermoelectric device, until reaching 22 ºC. We then modified the temperature to 37 ºC, 42 ºC and again to 37 ºC. In each epicardial temperature step, stabilization for at least two minutes was allowed before recording the electrograms under sinus node rhythm and subsequently under programmed pacing conditions.

Study parameters. Under sinus node rhythm in each of the two analyzed regions and at each of the different temperatures, we recorded ARI, measured from the instant of maximum negative slope of the QRS complex to the instant of maximum slope of the T wave (mean of 5 measurements). Under programmed pacing, we calculated the number of repetitive responses, counting one unit when following an extrastimulus one or more spontaneous depolarizations not directly produced by external pacing or generated by the sinus node appeared. Under programmed pacing, we determined CV from the activation maps obtained with a basic pacing cycle of 250 ms. Conduction velocity was calculated as the ratio between the distance separating two electrodes located in the direction perpendicular to the isochrones spaced at least 5 mm apart (one of them close to pacing) and the difference between their activation instants.

Statistical analysis. Data are reported as the mean ± standard deviation. Comparisons between two groups were made using the Student t-test for paired data, while single factor analysis of variance (ANOVA) for repeated measures was applied for comparisons of pooled data. Dependency between variables was evaluated using the chi-squared test with contingency tables. Linear regression between pairs of variables was performed with the least squares method. Statistical significance was considered for $p<0.05$. The SPSS® statistical package (SPSS Inc.) was used throughout, together with Microsoft Excel 2007.

3. Results

Activation-recovery interval (ARI). In the modified zone and with respect to baseline (37 ºC), ARI was seen to be prolonged in maximum hypothermia (137 ± 22 ms *vs* 111 ± 13 ms, $p<0.05$) and was shortened in hyperthermia (98 ± 13 ms *vs* 107 ± 16 ms, $p<0.05$).

Figure 2 shows the variation of ARI in the modified and unmodified zone. The localized thermal changes

produced ARI variations with respect to the basal situation in that zone. Likewise, differences with respect to the ARI of the distant zone were observed. The modifications in ARI in the unmodified zone were not significant ($p < 0.96$).

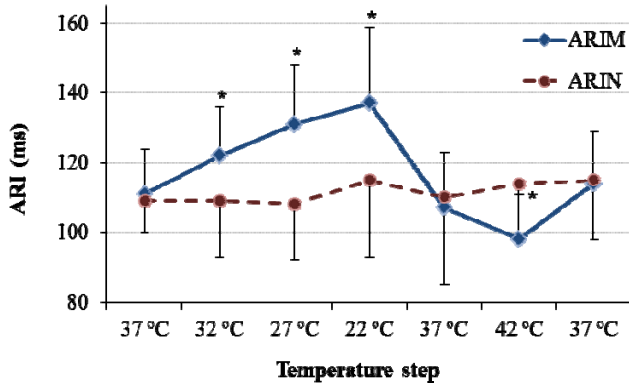

Figure 2. Evolution of the activation-recovery interval (ARI) in the modified (ARIM) and unmodified area (ARIN) in different temperature steps (n = 8). Significant differences compared to last step "37 ºC", *p <0.05.

In all cases the correlation between ARI in the modified zone and temperature fitted a straight line ($0.76 < |r| < 0.96$, $p<0.05$). On applying linear regression analysis to the overall points of all the experiments, the resulting straight line equation was: $ARIM = -2.01 \cdot TM + 184$ ($r=-0.64$; $p<0.0001$), where ARIM is ARI corresponding to the modified zone (in ms) and TM is the temperature in that zone (in ºC).

Conduction velocity (CV). The CV decreased significantly (*p<0.05) under hypothermia to 70 ± 17* cm/s at 22.2 ± 0.6 ºC from the basal value of 76 ± 17 cm/s at 36.8 ± 0.9 ºC, and increased under hypothermia to 79 ± 20* cm/s at 41.9 ± 0.1 ºC from 75 ± 21 cm/s at 37.3 ± 0.5 ºC. Figure 3 shows these results. In the zone not subjected to thermal modifications, the changes in CV did not reach statistical significance. Table 1 summarizes the numerical results obtained.

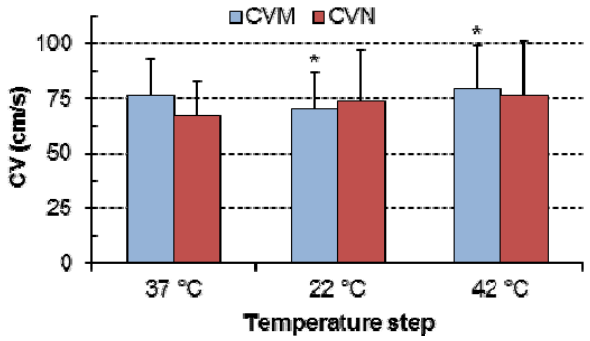

Figure 3. Mean values (n = 8) of conduction velocity in the modified (CVM) and unmodified area (CVN) during hypothermia (22 ºC) and hyperthermia (42 ºC) compared to the last step "37 ºC". Significant differences from baseline (*p<0.05).

Table 1. Temperature and conduction velocity in both zones: modified (TM, CVM) and unmodified (TN, CVN)(n=8). Significant differences in CVM versus the last step "37 °C" in the modified zone (*p<0.05).

Step	TM (°C)	CVM (cm/s)	TN (°C)	CVN (cm/s)
37 °C	36.8 ± 0.9	76 ± 17	37.7 ± 0.6	67 ± 16
32 °C	31.0 ± 2.1	72 ± 16	37.5 ± 0.7	66 ± 14
27 °C	26.7 ± 0.4	66 ± 19*	37.3 ± 0.6	68 ± 17
22 °C	22.3 ± 0.6	70 ± 17*	37.2 ± 0.6	74 ± 23
37 °C	37.3 ± 0.5	75 ± 21	37.3 ± 0.8	71 ± 22
42 °C	41.9 ± 0.1	79 ± 20*	37.6 ± 0.8	76 ± 25
37 °C	37.2 ± 0.2	75 ± 19	37.7 ± 0.4	72 ± 15

Presence of repetitive responses. After analyzing the signals obtained from the total extrastimulus tests performed, repetitive responses were observed in four hearts. A total of 39 repetitive responses were recorded. Of the overall repetitive responses, 23 occurred at basal temperature, 6 in some of the hypothermia steps, and 9 during hyperthermia. The chi-squared test revealed no dependency between the appearance of repetitive responses and locally induced hypothermia or hyperthermia ($p>0.3$). In no case did programmed pacing induce ventricular fibrillation.

4. Discussion

Electrophysiological parameters analyzed. The localized thermal modifications induced in the epicardium resulted in a proportional increase in ARI in the hypothermal zone. In turn, warming of the zone resulted in a decrease in ARI. In both cases the changes were confined to the interventional zone. The induced changes were seen to disappear on returning to the perfusion temperature – thus demonstrating the reversibility of the effect. Comparison between the zones showed that the observed changes – attributed to local temperature variations in the modified zone – significantly altered ARI. Within the analyzed interval, linear dependency was observed between epicardial temperature and the mentioned parameter. The method used to achieve gradual and controlled temperature modification was shown to be effective. We were able to generate electrophysiological heterogeneity in the ventricular myocardium, and this heterogeneity moreover increased in magnitude with the thermal gradient between zones. On the other hand, CV was found to decrease during hypothermia and increased with hyperthermia in the interventional zone. Among other factors, the latter phenomenon would be related to resting potential alterations, and therefore to phase zero of the action potential [8-9]. The thermally induced changes in refractoriness and CV may be considered to have resulted in changes in the propagation of activation, as seen in

previous studies [8,10-12].

Induction of ventricular arrhythmias. Localized thermal intervention upon the ventricular epicardium resulted in electrophysiological dispersion. Different studies have related this phenomenon to the induction of arrhythmogenic effects [4,13]. However, pacing with a short coupling interval [14] in proximity to the altered zone during thermal modification did not significantly modify the appearance of responses of this kind.

Thus, the changes induced in the epicardium were unable to generate arrhythmogenic conditions manifesting as repetitive responses or ventricular tachycardia. This could be attributed to insufficient ARI dispersion, related to a limited extent and depth of the thermal variations in the myocardium. The fact that we acted upon whole hearts subjected to constant coronary perfusion – in contrast to the situation in other studies [15] – could be the reason for this. Regarding the temperature-induced changes in the modified zone, the counterpoised nature of the variations in CV and refractoriness (indirectly associated to ARI) may have limited the changes in wavelength. This fact, and the limited depth of the affected region, could explain the absence of increased arrhythmia inducibility in our model.

In any case, in our electrophysiological alteration model, the local thermal changes did not evidence arrhythmogenic responses, very probably because of insufficient induced ARI dispersion.

5. Conclusion

Temperature changes induce heterogeneity in the myocardial substrate, resulting in dispersion of the electrophysiological parameters analyzed. Localized hypothermia prolongs ARI and reduces CV. In turn, local hyperthermia shortens ARI and increases CV. The magnitude of the effect depends on the degree of thermal variation achieved – a linear correlation being observed between temperature and ARI within the studied interval. The changes in turn are circumscribed to the thermally modified region and are reversible.

In our experimental model, although the local epicardial temperature variations significantly modified ARI and CV, the induced dispersion did not result in arrhythmogenic effects – probably because the encompassed tissue zone was not sufficiently extensive.

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