Modifications in the Activation Process during Ventricular Fibrillation by Local Hyperthermia

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Abstract

Because of its electrophysiological effects, temperature can influence the mechanisms of the ventricular fibrillation (VF). The aim of this work was to investigate, in experimental models, the effects of local hyperthermia on the activation frequency during VF, and its hypothetic influence on distant zones. The application of hyperthermia on a limited area of the epicardium was performed by a specific multielectrode-device, based on thick-film technology and Peltier effect.

A significant increase in the Dominant Frequency of the VF signal (DF) in the modified area was observed. Otherwise, the relative increments of DF in this zone and in the normothermic one, were correlated. In the model used, the effects of heating remitted after temperature returned to control value. Our results suggest that the activation process during VF is accelerated, in a reversible manner, by local hyperthermia, and this effect could affect normothermic zones.

1. Introduction

Recordings of epicardial electrical activity by means of multiple electrodes to register extracellular potentials, have provided valuable information about the characteristics of myocardial activation during ventricular arrhythmias, and therefore contributed to a deeper knowledge of mechanisms responsible of this malignant arrhythmia. There are many in vitro studies whose experimental protocol is based on isolated Langendorff-perfused animal hearts [1-7,12,13].

Some studies, making use of this technique, have previously analysed temperature influence in electrophysiological properties of myocardial tissue during basal activity and VF, among other factors. This studies have contributed with data concerning to variations in the action potential duration, the DF of signal spectrum, the depolarization wave wavelength or the cardiac tissue refractoriness during basal stimulation and arrhythmia has been [2-4]. Most of this works have been focused in studying the effects caused by hypothermia [5], but never those caused by hyperthermia, and mainly by global variations in preparation temperature [3], altering the perfusion solution (modifying the perfusion temperature or injecting cold tyrode directly inside of the ventricles). But it is also interesting to study what happens when neighbouring zones are affected by important temperature gradients [6]. For example, the unequal sensibility of different tissues under changes of temperature or the inhomogeneity in the modifications made to the preparation can simulate pathological conditions that contributes to the apparition of arrhythmias, as well as it happens in situations of myocardial ischemia or in some structural alterations produced by straightening, fibrosis or cicatrization of myocardial zones.

One hypothesis raised to explain activation during VF suggests the existence of fast activation zones with fibrillative conduction to the remaining myocardium [7,8]. If activation changes caused by local temperature variations are propagated to distal normothermic zones, this fact could corroborate this hypothesis.

Therefore, the analysis of the influence of local variations of temperature can bring forward useful data about the mechanisms that explain the initiation or the sustenance of arrhythmias.

2. Material and methods

Causing temperature variations in localized zones of the epicardium, while recording simultaneously the electrical activity, presents technical problems regarding to the methods and materials to use. In this work we have used a customized device to make possible this experimentation, allows us to register unipolar electrograms for the epicardial mapping while cooling or heating the same surface in which the recording is being taken [5]. After the system was developed and validated, it was applied to study the frequency content changes of VF signals with epicardial temperature.

2.1. Multielectrode

Two multiple electrodes were used for signal recording, a conventional plaque with 121 unipolar stainless steel electrodes (electrode diameter = 0.125 mm, inter-electrode distance = 1 mm), and a customized extraflat electrode joined at a heating device. For the construction of this multielectrode-device, thick-film microelectronic technology has been used. By means of silkscreen processes, flat electrodes are obtained by deposition of thin layers of conductor, dielectric and resistive materials, on a ceramic substrate. This technique provides good thermal conductivity. A Peltier Cell (thermoelectric cooler) and other accessory elements complete the system.

The schematic drawing in the Figure 1 shows the parts of the device developed as well as their distribution in a stratified disposition. In the surface that will be in contact with the heart, we have the following parts: multiple electrode in a ceramic substrate, Peltier Cell and aluminium refrigerator block. The matrix that conforms the multiple electrode contacts with the epicardium just in a small zone. A thin adhesive sheet of low thermal resistance joins the substrate and transmits the heat to the other face of the Peltier refrigerator. This one is connected to a power supply that drives the necessary current at each moment to obtain the desired temperature in the epicardial surface. The heat is pumped by Peltier effect toward the hot face of the thermoelectric refrigerator (in this case the surface in contact with the epicardium). The aluminium heat exchanger block is responsible of keeping constant the temperature of this face, enabling temperature lowering when the device is cooling the tissue (not used in this work). It has to be taken under consideration that the heart is continuously perfused with a nutritious liquid at 37ºC, which requires a continuous heat extraction flow. The dissipation block consists of a watertight cavity, manufactured for this purpose, with a refrigerating liquid circulating through it, under the action of a centrifugal pump in a closed circuit. The system is completed with two bipolar stimulation electrodes with lateral input (a lateral input o lateral inputs), and an extraflat thermocouple situated next to the matrix.

The electrode matrix consist of 4x4 electrodes of circular geometry, on an entire surface of 4.5x4.5 mm. The interceptor distance between electrodes is 1.5 mm. Figure 2 shows schematic views of this multiple electrode before its assembly in the final device. For its implementation several layers have been printed on a thin substrate of alumina (Al_2O_3). A conductive silver paste is used for printing the tracks which conduct the electrical signal up to a few contacts of a weldable material of Palladium-Silver. A blue polymeric ink acts as a dielectric, covering the whole surface except for the points in contact with the cardiac tissue, which form the electrodes in a strict sense. The process results in a multiple extraflat electrode which can be connected to the acquisition system by conventional welding in the corresponding terminations. The silver contacts have been chlorated by immersing the electrode in a constant current of ion chlorine solution, in order to stabilize its contact potential and to reduce the polarization impedance.

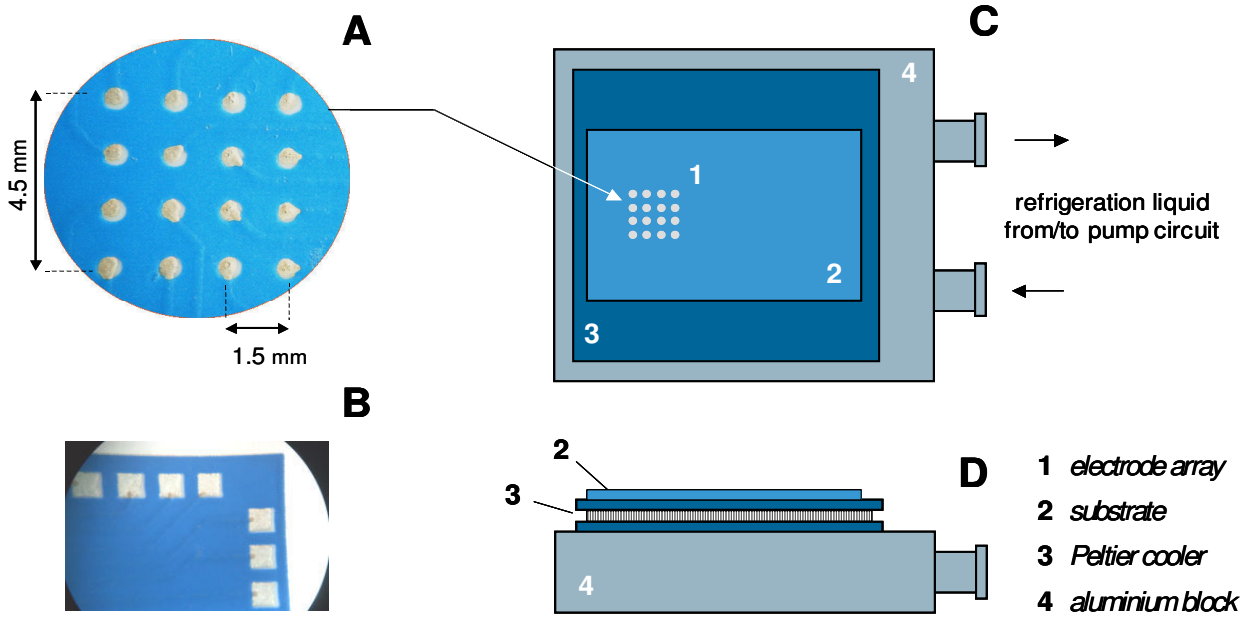

Figure 1. Microelectrode-device for heating and recording. A.- View of the electrodes at microscope. B.- Detail of soldering leads (microscope view). C.- Schematic top view. D.- Schematic lateral view.

2.2. Experimental preparation, protocol and data analysis

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]. The described device (16 unipolar electrodes) was positioned at the posterior wall of the left ventricle (modified zone, zMOD) and the standard one (121 unipolar electrodes) was positioned at the epicardial surface of the anterior wall of the same ventricle (normothermic zone, zNORM). This electrodes has been used to record electrograms during VF in 13 experimental models (California rabbits). After anesthesia with ketamine and heparinization, the hearts were removed and immersed in cold (4ºC) Tyrode solution. The composition of the perfusion fluid was (mM) 130 NaCl, 24.2 $NaHCO_3$, 4.7 KCl, 2.2 $CaCl_2$, 1.2 NaH_2PO_4, 0.6 $MgCl_2$, and 12 glucose. After isolation, the aorta was connected to a Langendorff system for perfusion of Tyrode solution at a pressure of 60 mmHg and a temperature of 37 ± 0.5ºC. Oxygenation was carried out with a mixture of 95% O_2 and 5% CO_2. The indifferent electrode was a 4x6-mm silver plaque located over the cannulated aorta. Recordings were obtained with a cardiac electrical activity mapping system (MAPTECH). The electrograms were amplified with a gain of 50-300, broadband (1-400 Hz) filtered, multiplexed and recorded. The sampling rate for each channel was 1kHz. The epicardial (modified and normothermic zone) and endocardial temperature were measured with miniaturized thermocouples and registered with a digital Fluke® thermometer.

VF was induced by pacing at increasing frequencies, maintaining coronary perfusion during arrhythmia. Five minutes after VF onset, the myocardial temperature was increased from 37ºC to 42ºC, maintained during five minutes at this value and then returned to basal value.

Welch's method was used to obtain the power spectrum of the signals [9,10] recorded at the end of each temperature step (last 4 seconds of 5 minutes), for each electrode. The spectral analysis was performed with data blocks of 4096 points (sampling rate = 1 kHz) and the Dominant Frequency was obtained for each electrode. Average values was calculated as activation process indicator [12,13]. Data processing was performed with software developed by our group for this purpose based on Matlab® [11]. Statistical data are presented as mean values ± standard deviation.

3. Results

Table 1 shows the average temperature and DF at both zones before, during and after hyperthermia. Differences between DF, at 37ºC, were not statistically significant (18.9±4.4Hz and 17.4±3.2Hz, normothermic and modified zone). 5 minutes after achieving hyperthermia, it was observed a significant increase in DF of the modified area, from basal value (DF=21.7±5.3Hz, $p<0.02$). The increment from the DF in normothermic area (DF=18.4±3.4Hz, $p<0.003$) was now statistically significant (Figure 3). In this zone the temperature showed no significant differences (37.2±0.6ºC). During hyperthermia, endocardial temperature of left ventricle remains stable (38.7±0.4ºC in opposition to 38.3±0.6ºC obtained for basal control). Otherwise, the relative increments of DF (from basal values) in the normothermic zone were correlated with the relative increments of DF in the modified zone ($r=0.63$, $p<0.02$) (Figure 4). In the experimental model used, the effects of heating remitted after temperature returned to control value (DF=17.9±4.2Hz, in modified zone).

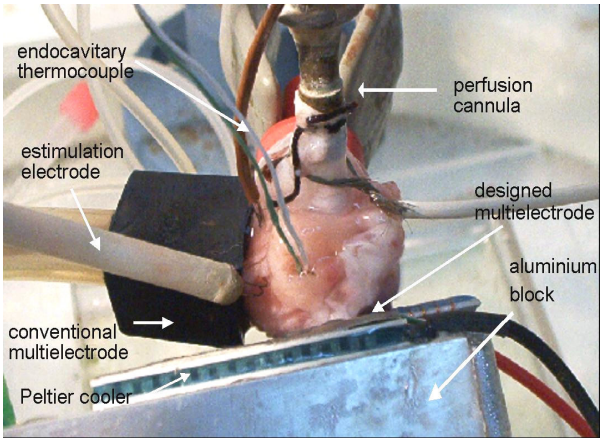

Figure 2. Experimental preparation. Isolated heart and electrodes situation.

		zNORM	zMOD
Tbasal	DF (Hz)	17.4±3.2	18.9±4.4
	T (ºC)	37.0±0.5	37.1±0.3
Hyperthermia	DF (Hz)	18.4±3.4	21.7±5.3
	T (ºC)	37.2±0.6	42.0±0.1
Tbasal AH	DF (Hz)	17.6±3.2	17.9±4.2
	T (ºC)	37.3±0.4	37.1±0.3

Table 1. Mean values of Temperatures and Dominant Frequencies. AH.- after hyperthermia.

4. Discussion and conclusions

The system developed to modify temperature in a limited area of the epicardial surface and recording the electrogram simultaneously works properly, as it allows us to fix temperature to a desired value in the contact

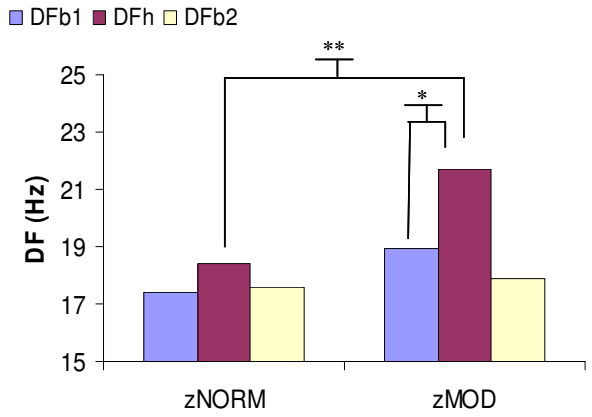

Figure 3. Dominant Frequencies at modified and normothermic zones. DFh.- during hyperthermia. DFb1.- at basal temperature. DFb2.- after hyperthermia. *p<0.02, **p<0.003

zone and set it without changes during the time interval required in our protocol, without causing temperature changes neither in distant epicardial zones nor in the inner side of the ventricle.

This device allow us to quantify the activation frequency on VF signals during local hyperthermia. It was found that heating (5ºC) a limited epicardial area results in a moderate acceleration of VF in modified zone. Correlation found between increments caused by hyperthermia on the DF of both zones (modified and normothermic) suggests the possibility of a distal propagation of the induced local acceleration. The arrhythmia persists during local epicardial heating, and effects on dominant frequency subside after suppressing epicardial heating.

In short, the results show that the activation frequency during VF is accelerated, in a reversible manner, by local hyperthermia and this effect could affect normothermic zones.

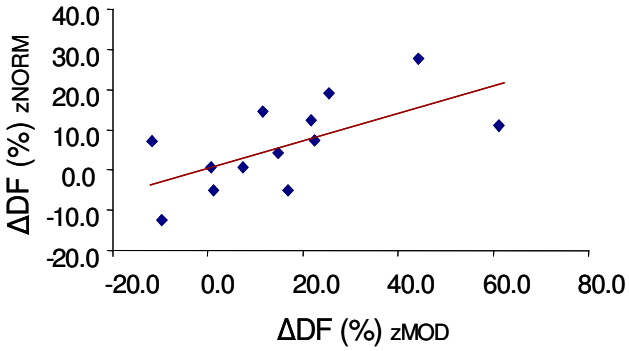

Figure 4. Correlation between relative increments of DF.

Acknowledgements

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