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Propagation and Electrical Impedance Changes due to Ischemia, Hypoxia and Reperfusion in Mouse Hearts

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***Abstract*—The purpose of this study is to quantitatively characterize major electrical markers of cardiac ischemia in normal mouse hearts to establish a set of baseline parameters for evaluation of genetically altered mouse hearts. Optical and electrical imaging techniques were coupled with impedance measurements to quantify changes induced by global ischemia. Optical and electrical mapping studies revealed the time course of conduction slowing and local inactivation during 30 minutes of ischemia or hypoxia. Measures of myocardial electrical impedance (MEI) were made during 30 and 120 minutes of global ischemia and proved to be qualitatively similar yet quantitatively distinct when compared to results reported from other mammals. The results of this study can now be applied in the analysis of genetically altered mouse hearts that are currently becoming available to help us understand cardiac death in disease.**

I. Introduction

DIABETES afflicts approximately 20 million individuals in the United States and according to the American Diabetes Association, 66% of these individuals will die of cardiac disease. Over the last 30 years mortality from cardiac disease has reduced by ~30% for nondiabetics, but has slightly increased for diabetics [1]. Our current research goals are to understand the unique cardiac substrate associated with diabetes and the mechanisms that make diabetics more vulnerable to cardiac death. Transgenic mouse models of diabetes and altered physiology associated with the disease provide valuable tools for reaching these goals. In order to correctly interpret the results from these transgenic mouse models, we must carefully characterize the mouse heart as a system and understand how it compares with other mammalian hearts, most importantly human hearts.

The purpose of this study was to quantitatively characterize the major electrical markers of cardiac ischemia and hypoxia in mouse hearts. Ischemia was targeted based on evidence of increased arrhythmias associated with ischemia in the setting of diabetes [2]. The primary objective was to provide quantitative measures of changes in propagation using optical and electrical imaging and changes in impedance using the four-electrode technique.

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II. Methods

A. Experimental Preparation

The Institutional Animal Care and Use Committee of the University of Utah reviewed and approved the experimental protocol for these studies. All mice (C57BL, male, 25-35g) were injected with heparin, followed by administration of sodium pentobarbital. The heart was then rapidly excised and Langendorff perfused via the aorta with Krebs-Henseleit buffer (in mM: 118.5 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 MgSO4, 0.5 EDTA, 1.2 KH2PO4, 2.5CaCl2, and 11 glucose), bubbled with 95% O_2 - 5% CO_2. Perfusion temperature, pressure and flow rate were constantly monitored and maintained at 37°C, 60 mmHg and greater than 1.0 ml/min respectively. The isolated hearts were positioned inside a custom made chamber to keep temperature at 37°C. Hearts were paced using small hooked Ag/AgCl wires (0.003 in diameter, California Fine Wire Inc.) attached to the anterior left ventricle for unipolar pacing. The heart was stimulated at levels just above threshold with duration of 2 ms and basic cycle lengths (BCLs) of 100 to 200 ms.

B. Optical Mapping

Hearts were perfused with 5μM concentration of the voltage sensitive fluoroprobe, di-4-ANEPPS in Krebs-Henseleit buffer for 5 minutes. Cytochalasin D (3μM) was continuously perfused to arrest the mechanical action. The optical mapping system developed in our laboratory features a 16 by 16 photo diode array with a preamplification system (Hamamatsu Corp., model C4675-103) [3]. After a minimum of 30 minutes of normal perfusion to stabilize the heart, baseline signals were recorded for 10 minutes followed by 30 minutes of global no flow ischemia and 30 minutes of reperfusion. Optical signals were recorded from the most centrally located 64 diodes forming an 8 by 8 array covering a 4mm by 4mm area of the anterior surface of the left ventricular free wall. Signals were recorded during normal sinus rhythm and during ventricular pacing from the epicardium in the center of the imaged area. Signals, 5 seconds in duration, were amplified, digitized at a 4000 Hz with 12-bit resolution and stored on a Macintonish G4 computer. The activation time was computed as the time of the maximum value of the time derivative during the upstroke of the signal. Total activation time (TAT) was calculated as the latest activation time minus the earliest activation time within the imaged area. Graphic visualization of activation times and potentials was achieved with map3d

software (http://www.sci.utah.edu/ncrr/software/map3d.html).

C. Electrical Imaging

A 64-lead "cage" electrode array was fabricated from 0.007 in diameter silver wire with 1.94 mm horizontal and 1.29 mm vertical interelectrode spacing (See Fig. 1). The array was slipped over the heart making contact with the ventricular free walls via a reservoir of fluid maintained at 37°C surrounding the heart [4]. After 30 minutes of normal perfusion to stabilize the heart, baseline signals were recorded for 10 minutes followed by 30 minutes of hypoxia (PO_2 < 20 mmHg) and 30 minutes of reperfusion. Unipolar electrograms were recorded and analyzed using the same equipment and methods as described for the optical signals. However, activation time from the unipolar electrograms was computed as the time of the minimum value of the time derivative during the QRS interval. [5].

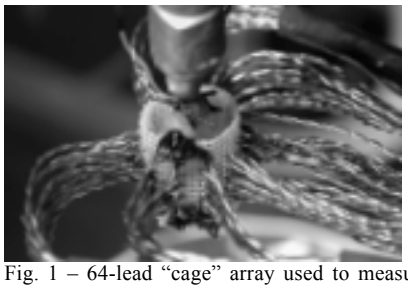

Fig. 1 – 64-lead "cage" array used to measure unipolar electrograms from the ventricular surface of mouse hearts.

D. Myocardial Electrical Impedance (MEI)

Measurement of MEI was performed using the four-electrode technique [6] at 1000Hz in isolated hearts. The probe consisted of a linear array of four platinum electrodes, with an inter-electrode spacing of 1 mm, and was placed against the left ventricular free wall. An alternating current of 10μA at 1000 Hz was applied through the outer pair of electrodes and the resulting potential difference was recorded from the inner pair of electrodes. After stabilization, the effects of no flow ischemia on MEI were analyzed. The effects of ischemia and the gap junction blocker, heptanol (1μM), on MEI were also analyzed.

E. Statistical Analysis

The data are expressed as the mean ± the standard deviation. The student's (paired or unpaired) t test was used to compare the mean difference between groups and a p value lower than 0.05 was considered to be significant.

III. Results

A. Optical Mapping

Total activation time of the imaged area during normal sinus rhythm under control conditions was 4.4 ± 2.4 ms (n=5). TAT sharply increased due to the onset of global ischemia reaching 7.3 ± 3.9 ms (p<0.05) and 14.4 ± 6.8 ms (p<0.001) after 2 and 3 minutes, respectively. TAT decreased dramatically after reperfusion. TAT decreased dramatically after reperfusion. TAT was 7.5 ± 4.6 ms (p<0.05), 6. 8 ± 6.7 ms (p>0.05) and 4.9 ± 3.6 ms (p>0.05) after 1, 2 and 3 minutes of reperfusion (See Fig. 2).

During ventricular pacing, TAT after 2 minutes of reperfusion was the same as that of pre-ischemia (13.5 ± 7.2 ms vs. 12.0 ± 2.6 ms, p>0.05). Four out five hearts could not be captured during ventricular pacing after the first minute of ischemia.

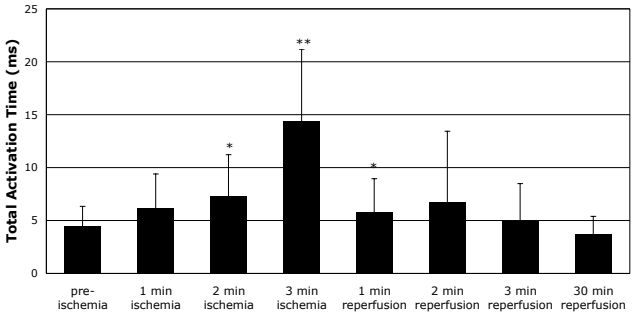

Fig. 2 – Total activation time as measured during normal sinus rhythm on the left ventricular free wall of mouse hearts using optical mapping techniques as a function of time during global ischemia and reperfusion. (*: p<0.05, **: p<0.001 vs. pre-ischemia, n=5)

B. Electrical Imaging

Total ventricular activation time during normal sinus rhythm was measured to be 3.5 ± 0.8 ms from the cage electrode array. TAT increased an average of 18% after 2 minutes of hypoxia. In 40% of the hearts, additional increases in TAT were small and the hearts showed modest recovery after 30 minutes of reperfusion. In the remaining 60% of the hearts TAT continued to increase during 30 minutes of hypoxia and did not fully recover after 30 minutes of reperfusion.

The rapid change in potential associated with the QRS interval of the unipolar electrogram is a manifestation of the local tissue depolarization. Changes in the slope of the downstroke of the electrograms tracked during hypoxia gave a dynamic indication of changes in the local tissue activation properties. During the first 10 minutes of hypoxia, there was a decrease in the slope of the electrograms of ~40%. In 60% of the hearts, this initial decrease was followed by a more rapid secondary decrease of approximately 20%. Like TAT, the slope of the electrograms does not recover to control values after 30 minutes of reperfusion in a majority of the hearts.

C. Myocardial Electrical Impedance

Measures of MEI were made during 30 minutes of global ischemia followed by 30 minutes of normal reperfusion. In these studies MEI rapidly increased by 50% within the first 5 minutes of ischemia and then plateaus with very gradual increases over the next 20 minutes. Values of MEI returned to baseline after 1-2 minutes of reperfusion.

Fig. 3 shows normalized measures of impedance as a function of time during 120 minutes of ischemia. In this figure, the initial increase of 50% during the first 5 minutes (marked by first dashed line) can be seen. In addition, after

approximately 40 minutes of ischemia there is a secondary rise in impedance of approximately 10%.

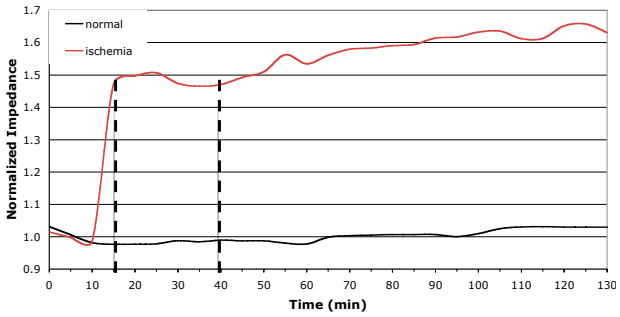

Fig. 3. Effects of ischemia on MEI. Global ischemia begins after 10 minutes of recordings at normal conditions. (normal group n = 2; ischemia group n=4

To investigate the possibility that the secondary rise in MEI is of similar magnitude to that caused by gap junction closure, the gap junction blocker, heptanol, was added to the perfusate 15 minutes prior to the onset of ischemia in a subset of experiments. Fig. 4 shows the initial rise of MEI of approximately 10% in response to the heptanol followed by the greater change due to the onset of ischemia. Fig. 4 shows the results of these studies. MEI increased approximately 10% in response to the heptanol. Both, the hearts perfused with heptanol and the control hearts showed a 50% increase in MEI after 5 minutes of ischemia. The control hearts, however, demonstrated a secondary rise after

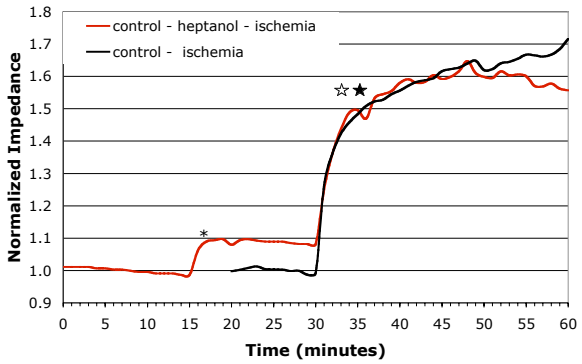

Fig. 4 - Effects of heptanol and global ischemia on MEI. (* indicates $p<0.05$ control vs. 1 minute of heptanol perfusion, ☆ indicates $p<0.05$ for 31min vs. 30min of effects of heptanol and ischemia. ★ indicates $p<0.001$ for 11min vs. 10min of effects of ischemia only, n=7.)

approximately 30 minutes of ischemia where the heptanol-perfused hearts did not.

IV. Discussion

The results of this study are very important for establishing a baseline characterization of global ischemia in the mouse model. These results provide the necessary unique baseline parameters for conduction slowing and local inactivation using total activation time and the slope of the action potential and unipolar electrogram in a sufficient way to distinguish normal from abnormal propagation due to ischemia. MEI measurements provided insight into the time course of impedance changes in the mouse heart during ischemia and proved to be qualitatively similar but quantitatively distinct from findings reported for other mammals.

We found that TAT for a 16 mm^2 area of the LV was longer than the total ventricular activation time from the cage electrode. These results are consistent with previous results from our laboratory and we suspect that the use of di-4ANEPPS and Cytochalasin D for optical mapping is responsible for the slowing seen in the optical mapping studies as others have reported [7]. In addition, we found 30 minutes of ischemia to be reversible in the optical mapping studies, consistent with similar results reported in rat [8]. However, in the electrical mapping studies 30 minutes of hypoxia, for a majority of the hearts proved to be nonreversible. Studies are underway to investigate the mechanisms of these differences in the effects of ischemia versus hypoxia.

Measures of MEI during 120 minutes of ischemia are qualitatively very similar to those reported in pig [9]. Both show a rapid rise within the first 5 minutes of ischemia followed by a plateau and then a secondary rise after 35 to 40 minutes. Quantitatively, the rise in MEI measured in mouse for the first phase was larger than that measured in pig (50% versus 10%, respectively). In mouse the secondary rise is only on the order of 10% compared with 20% in pigs and dogs [9]. The magnitude of changes in MEI values in response to the gap junction blocker heptanol were similar to those shown in rat [8] and are of the same amplitude as those associated with the secondary increase in MEI during prolonged ischemia, thus suggesting this secondary rise reflects the timing of gap junction closure during prolonged ischemia.

These findings can now be applied in the analysis of genetically altered mouse hearts that are currently becoming available to help us understand cardiac death in disease states like diabetes.

V. References

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