Susceptibility of Isolated Rabbit Hearts with Various Left Ventricular Mass to Short Ischemic Periods

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Abstract

Increased left ventricular (LV) mass leads to higher sensitivity of heart to ischemic injury. This study was focused on comparison of susceptibility of rabbit hearts with different LV mass to short period of ischemia and its modification in consequence of repetition of short ischemic periods. Although higher count of arrhythmias was observed in hearts with higher LV mass during the first ischemia, decrease of total number of arrhythmias and their later onset during repeated ischemic periods were more pronounced in these hearts in comparison with hearts with low LV mass.

1. Introduction

Isolated heart retrogradely perfused according to Langendorff represents "golden standard" among experimental cardiology methods. Although various species of laboratory animals may be used for this model, rabbit heart is one of the most popular ones for studying of cardiovascular system. Rabbit heart is the optimal compromise between high similarity of cardiovascular parameters with humans which is typical for big animal models and easy breeding and low cost of small laboratory animals.

Rabbit myocardium also shows high level of similarity with human myocardium - in basic electrophysiology parameters [1], ionic channels distribution, process of repolarization, and calcium handling [2]. Moreover, rabbits are highly sensitive to stress which leads to hypertrophy of heart and myocardial coronary vasoconstriction [3]. Therefore, rabbit heart represents an ideal model for correlation study between LV mass and susceptibility to ischemia.

The first aim of this study was to compare rabbit hearts with low (group L) and high (group H) left ventricular weight to heart weight ratio (LVW/HW ratio) as their susceptibility to repeated short periods of global ischemia is concerned. The second aim was to search for biochemical parameter able to predict discrete changes at myocardial membrane resulting from ischemic insult.

2. Methods

2.1. Isolated heart preparation

All experiments were carried out according to the guidelines for animal treatment approved by local authorities and conformed to the EU law. Seventeen adult New Zealand rabbits were included in this study. Animals were anesthetized by intramuscular application of xylazin (2 mg/kg) and ketamine (60 mg/kg). The heart was excised and placed into a bath containing cold Krebs–Henseleit solution (4 °C) and the aorta was cannulated. The heart was then fixed to a modified Langendorff set-up [4] and perfused at constant pressure (80 mmHg) with Krebs-Henseleit solution (NaCl, 118 mM; $NaHCO_3$, 24 mM; KCl, 4.2 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.25 mM; glucose, 5.5 mM), continuously aerated with 95% O_2 and 5%. The temperature was maintained constant at 37 °C.

After 30 minutes of stabilization, heart underwent 10 minutes of ischemia and 10 minutes of reperfusion, once or three times (for protocol see Fig.1).

Three orthogonal electrograms were recorded using touch-less bipolar electrode system (three pairs of Ag-AgCl disc electrodes) placed in the wall of the bath, using USB PC card (National Instruments), with sampling rate of 2 kHz and resolution of 12 bits. The coronary effluent was drained from the bath during the experiment via overflow and measured.

Immediately after the end of last reperfusion, the heart and its left ventricle were weighted and LV wall thickness was measured.

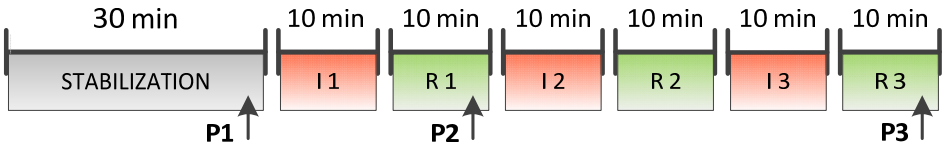

Figure 1. Experimental protocol; I - ischemia, R - reperfusion; P1, 2, 3 - collection of perfusate samples.

The body weight to heart weight ratio, LV weight to heart weight ratio and LV weight to LV wall thickness ratio were calculated. The animals were then distributed in two groups according LVW/HW ratio, i.e. with low (L) and high (H) LVW/HW ratio (below or equal to and above 0.57, respectively).

2.2. Electrophysiology data analysis

Susceptibility of hearts with low and high LV mass to repeated short periods of ischemia was assessed by both electrophysiological and biochemical parameters. Electrophysiology vulnerability was considered by counting of the total number of ventricular premature beats (VPBs), their severity (singles, salvos, ventricular tachycardia) and time of their onset.

The heart rate (HR) was measured at the end of each 5th minute during all experiment. The results were then normalized to the end of stabilization period (100%).

Differences in VPBs number between the groups were assessed by Mann-Whitney U test. The results of HR were expressed as mean ± SEM. Standard parametric and nonparametric descriptive statistics (mean, median, range) were done for each phase. The differences between consecutive phases of the experiment were analyzed by Student's paired t-test. Differences between individual phases of each experiment and its stabilization phase were analyzed by one-sample t-test. Differences between two experimental groups were analyzed by Student's unpaired t-test. P values were calculated, $P < 0.05$ was considered significant. Analyzes were performed using GraphPad Prism® 5 (version 5.01, GraphPad Software, Inc., San Diego, CA).

2.3. Biochemical data analysis

The creatine kinase (CK), lactate dehydrogenase (LDH), lactate, and 4-hydroxynonenal (HNE, marker of lipoperoxidation) were measured in samples of perfusate collected at the end of stabilization period, at the end of the first reperfusion and at the end of the third reperfusion (see Fig.1). Cardiac enzymes (CK, LDH) and lactate were determined by commercial kits (Erba Lachema, Czech Republic and Biovendor, Czech republic, respectively). The results were expressed in μkat/l and mmol/l (CK, LDH and lactate, respectively). HNE determination was performed according to Kinter [5] with slight modification. Briefly, the method was based on derivatization by dinitrophenylhydrazine, extraction to hexane and HPLC analysis with UV detection. The concentration of HNE in perfusate was expressed in nmol/l.

The values of biochemical markers measured at the end of three phases of experiment (stabilization the 1st and the 3rd reperfusion) were then compared using Wilcoxon signed-rank test at the 5% significance level.

3. Results

3.1. Basic characteristic of data sets

There were no significant differences between groups in body weight (2.8 ± 0.4 kg and 2.9 ± 0.4 kg in group L and H, respectively), heart weight (10.3 ± 2.7 g in group L and 10.4 ± 2.7 g in group H, respectively) and LV weight (5.8 ± 1.4 g in group L and 6.1 ± 1.7 g in group H, respectively). LVW/HW ratio was the only parameter in which groups differed significantly (0.53 ± 0.03 in group L and 0.61 ± 0.03 in group H, respectively). All measured parameters and calculated ratios are summarized in Table 1.

3.2. Electrophysiology data

The total number of VPBs during the first ischemia was higher in group H (282 ± 12.6) with predominant grade in salvos (193 ± 9.4) and onset in the 6th minute in comparison with lower number of VPBs in group L (116 ±7.8) with dominant singles (93 ± 7.1) and onset in the 5th minute. Both groups showed decrease of total number of VPBs and delay of onset of arrhythmias during the second ischemia (to 71 % and the 7th minute in group H and to 11 % and 7th minute in group L, respectively). Example of electrogram with VPBs is shown in Figure 2.

The dominant form of arrhythmias remained unchanged in both groups. During the third ischemia the decrease of total number of VPBs and delay of onset of arrhythmias continued in group H (to 10% and the 9th minute, but predominant arrhythmias type was VT). In group L, total number of VPBs during the third ischemia increased to 39 % of total number of VPBs observed in the first ischemia and their onset remained in the 7th minute. Similarly to group H, predominant arrhythmias progressed to more severe type, i.e. salvos (see Table 2 and Figure 3).

Table 1. Basic characteristics of data sets.

Group	BW (kg)	HW (g)	LVW (g)	Wall thickness (mm)	HW to BW ratio	LVW to HW ratio	Gender distribution
L	2.8 ± 0.4	10.3 ± 2.7	5.8 ± 1.4	6.1 ± 1.8	0.37 ± 0.06	0.53 ± 0.03	6M:2F
H	2.9 ± 0.4	10.2 ± 2.7	6.1 ± 1.7	7.1 ± 0.8	0.36 ± 0.05	0.61 ± 0.03**	6M:2F

Values are means ± SEM; ** P < 0.001; BW – body weight, HW–heart weight, LVW – left ventricular weight, HW to BW – heart weight to body weight ratio, LVW to HW – left ventricle weight to heart weight ratio; M – male, F – female

Table 2. Total number and forms of VPBs and their onset

Values are means ± SEM; I – ischemia; n – number of animals; I 1, 2, 3 – ischemia; VT – ventricular tachycardia; VPBs – ventricular premature beats

Group	Phase	n	Singles	Salvos	VT	Total VPBs	Onset of arrhythmias
L	I1	6	93 ± 7.1	21 ± 2.8	2 ± 0.3	116 ±.7.8	5th minute
H		6	56 ± 2.4	193 ± 9.4	33 ± 3.3	282 ± 12.6	6th minute
L	I2	6	13.0 ± 1.0	0	0	13.0 ± 1.6	7th minute
H		6	20 ± 1.7	153 ± 11.1	31 ± 2.8	204 ± 1.6	7th minute
L	I3	6	20 ± 1.2	25 ± 1.9	0	45 ± 3.5	7th minute
H		6	1 ± 0.3	6 ± 0.9	20 ± 2.1	27 ± 2.3	9th minute

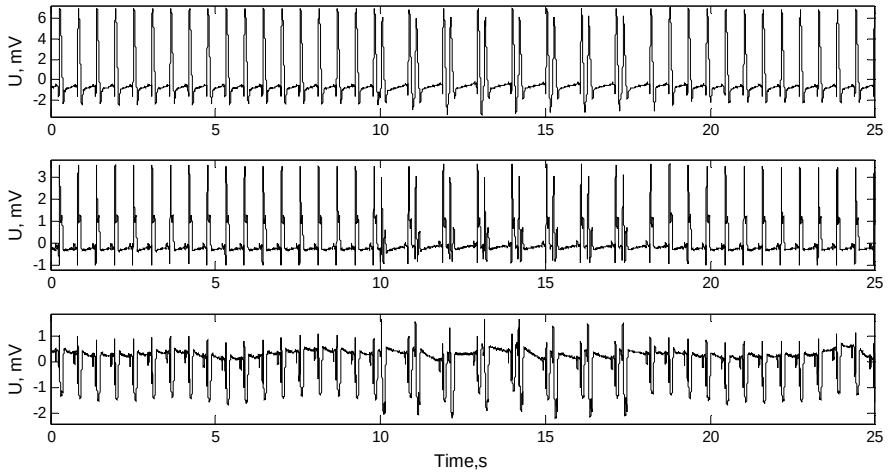

Figure 2. Example of orthogonal electrograms recorded simultaneously during the first ischemia.

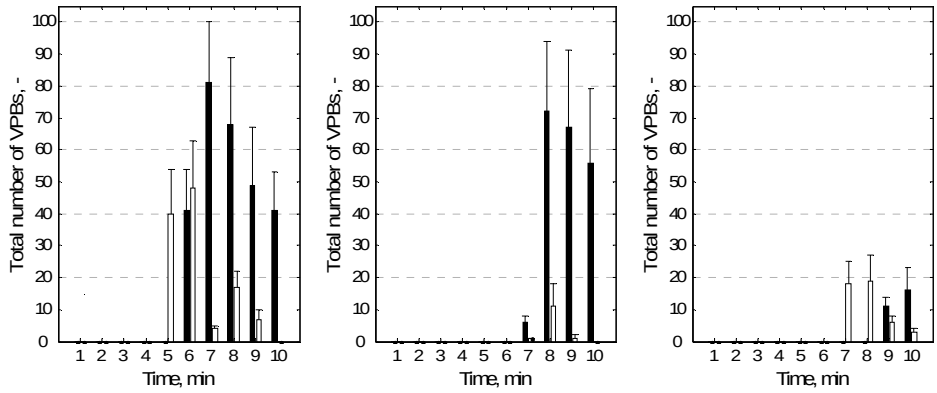

Figure 3. Distribution of VPBs over the first, the second and the third ischemia (left, middle, and right panel, respectively). Results for L and H groups are depicted with black and white boxes, respectively.

There were no arrhythmias in any of reperfusion periods. HR decreased in ischemic periods and mostly restored during reperfusions in both groups. More pronounced decrease of HR in the first ischemia was observed in group L, following reperfusion and the second ischemia did not show any significant difference between the groups, but better recovery in the second ischemia was present in group H. The changes in HR in the third ischemia and reperfusion were very similar between the groups (see Figure 4).

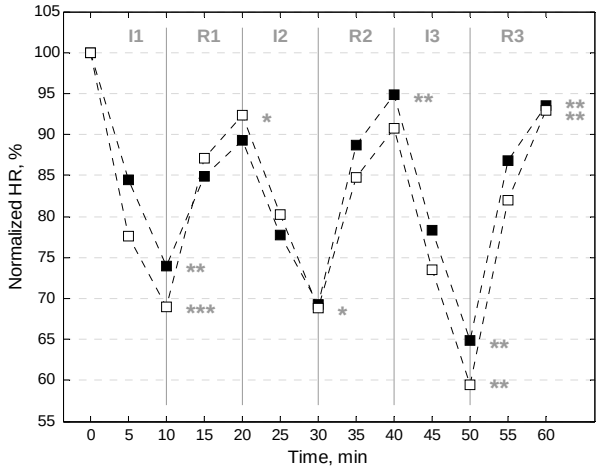

Figure 4. Mean values of normalized HR for L and H group (depicted with black and white markers, respectively). HR values significantly different from those in previous experimental phase are depicted with *, **, and *** for P<0.05, P<0.001, and P<0.0008, respectively (Student's paired t-test). I - ischemia, R - reperfusion.

3.3. Biochemical data

Group H showed increase in CK, LDH, lactate, and HNE after the first reperfusion as compared to the stabilization period. Then the values of these parameters were restored approximately to their stabilization levels after the third reperfusion. In group L, the changes were similar, but very discrete.

The most considerable changes were determined for LDH and HNE (marker of lipoperoxidation) in group H. There were significant differences in values of LDH in the first reperfusion and the third reperfusion (1.58 ± 0.19 μkat/l and 1.50 ± 0.17 μkat/l, respectively) and in values of HNE in stabilization period and the first reperfusion (19.9 ± 6.9 nmol/l and 25.3 ± 7.7 nmol/l, respectively).

4. Conclusion

We can conclude that in our experimental setup the hearts with higher LV mass were more susceptible to ischemia, as evaluated by induced arrhythmias; however, preconditioning effect resulting from previous ischemia was more pronounced in these hearts than in the hearts with low LV mass.

HNE seems to be promising marker in prediction of discrete ischemic injury on the membrane of cardiomyocytes. Its increased release in this model might denote enhanced lipoperoxidation and consecutive changes in gating of specific ionic channels on sarcolemma resulting in increased incidence of arrhythmias.

Acknowledgements

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