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A protocol to study *ex vivo* mouse working heart at human-like heart rate

Han-Zhong Feng, Jian-Ping Jin*

Wayne State University School of Medicine, Physiology Department, 540 East Canfield Street, Detroit, MI 48201, USA

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ABSTRACT

Genetically modified mice are widely used as experimental models to study human heart function and diseases. However, the fast rate of normal mouse heart at 400–600 bpm limits its capacity of assessing kinetic parameters that are important for the physiology and pathophysiology of human heart that beats at a much slower rate (75–180 bpm). To extend the value of mouse models, we established a protocol to study *ex vivo* mouse working hearts at a human-like heart rate. In the presence of 300 μM lidocaine to lower pacemaker and conductive activities and prevent arrhythmia, a stable rate of 120–130 bpm at 37 °C is achieved for *ex vivo* mouse working hearts. The negative effects of decreased heart rate on force-frequency dependence and lidocaine as a myocardial depressant on intracellular calcium can be compensated by using a higher but still physiological level of calcium (2.75 mM) in the perfusion media. Multiple parameters were studied to compare the function at the human-like heart rate with that of *ex vivo* mouse working hearts at the standard rate of 480 bpm. The results showed that the conditions for slower heart rate in the presence of 300 μM lidocaine did not have depressing effect on left ventricular pressure development, systolic and diastolic velocities and stroke volume with maintained positive inotropic and lusitropic responses to β-adrenergic stimulation. Compared with that at 480 bpm, the human-like heart rate increased ventricular filling and end diastolic volume with enhanced Frank-Starling responses. Coronary perfusion was increased from longer relaxation time and interval between beats whereas cardiac efficiency was significantly improved. Although the intrinsic differences between mouse and human heart remain, this methodology for *ex vivo* mouse hearts to work at human-like heart rate extends the value of using genetically modified mouse models to study cardiac function and human heart diseases.

1. Introduction

Genetically modified mice are widely-used as animal models to study human heart function and diseases. Despite having plausible similarities in cardiac anatomy, development and physiology [1,2], the mouse and human hearts, however, have differences that need to be considered when interpreting studies of mouse hearts to understand human cardiac function. The adult mouse heart is small (~100–200 mg in weight) and fast beating (400–800 beats per min, bpm), whereas the adult human heart weighs 250–300 g, works at 60–70 bpm at rest and up to 180–200 bpm during exercise [2]. Implicating a difference in calcium homeostasis, the hearts of large, but not small, mammals display a positive force-frequency effect [3] while cardiomyocytes of small mammals with high heart rates have greater sarcoplasmic reticulum (SR) Ca^{2+}-ATPase activity [4] and plasma membrane Na/Ca exchanger density [5]. The contractile proteins, especially myosin isoenzymes, are also different in mouse and human hearts [6–8]. Although some of these differences are intrinsic and unavoidable, mouse hearts remain a highly valuable experimental system in biomedical research and continuing technical improvement can maximize their application and impacts.

Isolated *ex vivo* working heart [9], is a powerful technique with many unique advantages over *in vivo* echocardiography [10] or Langendorff retrograde perfused heart [11] in the study of cardiac function. The working heart preparation is in a physiologically ejecting mode with *in vivo*-like dynamic changes in the left ventricular pressure and volume. The development of ventricular pressure and volume can be directly measured in real time using aortic pressure transducer and pressure-volume (P-V) catheter inserted into the left ventricular chamber. Temperature, preload, afterload, and heart rate are precisely controlled and readily adjustable. Left ventricular stroke volume and cardiac output can be directly and accurately measured. Systemic neurohumoral interferences are excluded. Drugs and other treatments such as pH, electrolytes and oxygen in the perfusant can be readily added or removed. Cardiac efficiency can be calculated from cardiac output, left ventricular (LV) systolic and kinetic integrals, and oxygen

Abbreviations: HR, heart rate; ISO, isoproterenol; LV, left ventricle; LVP, left ventricular pressure; RA, right atrium

* Corresponding author.
E-mail address: jjin@med.wayne.edu (J.-P. Jin).

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consumption measured from coronary effluent. Electrocardiograph (ECG) can also be recorded in *ex vivo* working hearts [12–14].

Ex vivo mouse working hearts are normally studied at heart rate of 400–600 bpm, mimicking their physiological rate *in vivo*. However, this fast heart rate imposes a major limitation to its use in the study of human cardiac function and diseases. For example, LV development pressure (LVDevP), ± dP/dt and cardiac power (the product of LVDevP and cardiac output) decline markedly when heart rate increases, concurring with an increase in LV end diastolic pressure (LVEDP) [13]. The high beating rate of mouse heart also produces shorter systolic and diastolic time durations with kinetic parameters significantly different from that of human hearts. Fast heart rate reduces ventricular end-diastolic volume, restricting the evaluation of Frank-Starling response in mouse working hearts. Taking the advantage that the heart rate of *ex vivo* working heart can be technically controlled, our present study developed a protocol to study mouse working hearts at a human-like heart rate in order to overcome some of the above limitations and extend its application in kinetic studies and in the investigations of human heart diseases and failure.

2. Materials and methods

2.1. Animals

Wild type male and female C57BL/6 mice of 4–5 months old were used in this study. All animal procedures were approved by the Institutional Animal Care and Use Committee of Wayne State University and were conducted in accordance with the Guiding Principles in the Care and Use of Animals, under the guidelines of the Council of the American Physiological Society.

2.2. Setup of ex vivo mouse working heart preparations

Preparation of isolated mouse working hearts was done using the protocol described in our previous studies [14]. Briefly, mouse was heparinized (100 U, i.p.) 30 min before anesthesia with pentobarbital (100 mg/kg, i.p.). After opening the chest, the heart was excised rapidly together with lung, trachea and thoracic aorta. The heart was submerged in Kreb's buffer at room temperature and carefully dissected with the large vessels attached for aortic cannulation. Within 2 min after the opening of the chest, Langendorff retrograde perfusion of the heart using Kreb's solution at 37 °C was established from the aortic cannula connected to an 80 mmHg perfusion system (Radnoti, US).

The Kreb's perfusion medium was modified from Krebs-Henseleit bicarbonate buffer, containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.25 mM $MgSO_4$, 2.25 mM or 2.75 mM $CaCl_2$, 0.32 mM EGTA, 2 mM pyruvate, and 15 mM D-glucose, equilibrated with 95% O_2–5% CO_2. $NaHCO_3$ was added to adjust the pH to 7.4 at 37 °C. The perfusion medium was filtered through a 0.45-μm membrane and was not circulated for reuse.

The pulmonary vein and pulmonary artery were then cannulated for switching the heart to working mode. A 1.2F P-V catheter with 3.5 mm electrode spacing (Transonic Scisense Inc., Canada) was inserted into the left ventricular chamber through a track made at the apex using a 30 g needle to record the intraventricular pressure and volume. A pair of lab-made pacing electrodes was placed on the surface of right atrium to provide electrical stimulation from an isolated stimulator (A365, World Precision Instrument, USA). An electrode made from a needle of 7-0 surgical suture was placed pericardial at the apex and connected to an ECG recording system (AD Instrument) together with a reference electrode placed inside the pulmonary vein-left atria cannula. A pressure transducer was place in the path of aortic cannula to record aortic pressure. A pair of copper wires with one wire attached with an iron clip was placed under the outlet of aortic flow to record the aortic output in calibrated drops in real time. Another pair was placed under the outlet of pulmonary flow to record coronary flow. The preload was set as the hydraulic height between the surface of the perfusant in the reservoir connected to the pulmonary vein and the left atrium. The afterload was set as the hydraulic height between the outlet of the aortic outflow track and the left ventricle. The signals of aortic pressure, LV pressure and volume, ECG, aortic output and coronary flow were digitized *via* an AD interface (16 Channels Powerlab, AD Instrument) and continuously collected using Chart5 computer software (AD Instrument) for later analysis.

After all cannulation and electrodes were setup, the heart was switched to ejection mode by turning on the left ventricular preload flow. Preload was set at 10 mmHg and afterload at 55 mmHg as the baseline condition. After functional stabilization was achieved, LV end systolic pressure-volume relationship (ESPVR) was measured by pressing the aortic outflow tubing to temporarily increase resistance and recording functional changes during the returning of afterload to normal after the tubing pressing was released. Left ventricular end diastolic pressure-volume relationship (EDPVR) was measured by lowering preload from 10 to 5 mmHg. Frank-starling relationship was tested by altering preload between 5 and 20 mmHg.

2.3. Modified steps for the study of mouse working heart at human-like heart rate

Illustrated in Fig. 1A, we modified the mouse working heart protocol to explore conditions for the study of cardiac function at slower and human-like heart rate. Functional measurements were first performed at the standard heart rate of 480 bpm [14]. Cardiac function was then measured at 420 bpm and 360 bpm pacing, and at the intrinsic sinus rate of ~230 bpm (37 °C) without external pacing. A series of accumulated doses of lidocaine (VETONE) at 100, 200, and 300 μM were added to the preload reservoir to prevent arrhythmia and to decrease the intrinsic heart rate by inhibiting atrial rate with minor decreasing of A-V conduction [15]. Each dosage was maintained for 5 min to obtain stable functional measurements. Pacing was tested at various frequencies to identify a condition that produced a stable human-like rate of ventricular beating.

Considering that high doses of lidocaine has a cardiac depressant property by affecting intracellular calcium [16] and lower heart rate diminishes the effect of positive force-frequency relationship [17], we tested a compensation by increasing the Ca^{2+} concentration in the perfusion media. $CaCl_2$ was increased from 2.25 mM to 2.75 mM, a concentration near the higher end of the physiological level in plasma. At the end of the protocol, 2 nM isoproterenol (ISO) was applied at 300 μM lidocaine to test β-adrenergic response.

2.4. Data analysis

All quantitative data are presented as mean ± SEM. Student's *t*-test was performed for comparisons between means and ANOVA was used for comparison between curves. $P < 0.05$ was used to establish the level of significance.

3. Results

3.1. Increasing Ca^{2+} in perfusion media compensates for ex vivo mouse working heart function at decreased heart rates

Perfused with the Kreb's solution normally used in working heart studies containing 2.25 mM $CaCl_2$ (Fig. 1), LV stroke volume of *ex vivo* mouse working heart increased when heart rate was decreased from 480 to 360 bpm but then decreased at heart rate of ~260 bpm with decreased systolic and diastolic velocities (Table 1). Countering the decrease in intracellular Ca^{2+} at slower heart rate based on the positive force-frequency relationship of mammalian cardiomyocytes [17], the increase of Ca^{2+} concentration in Kreb's solution to an upper physiological level of 2.75 mM effectively compensated for the reduction of

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