Increased Heart Rate Reduced Crossbridge Formation in Beating Rat Whole Heart

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Abstract: - Heart rate is one of the determinant factors of cardiac performance. However, there is no direct evidence for the effect of heart rate on the actin-myosin interaction. Purpose: To test the effect of heart rates on actin-myosin interaction in hearts with X-ray diffraction analysis using SPring-8. Methods: Seven isolated isovolumically contracting rat hearts were mounted so that the X-ray beam (15.0 keV) passed the subepicardial region. We recorded X-ray diffraction images and LV pressure at heart rates of 120 and 300bpm under 0 mmHg end-diastolic pressure. Between two different heart rates, we compared 1) the amount and the duration of actin-myosin interaction from the intensity ratio reduction of inner (1,0) and outer (1,1) X-ray diffraction, and 2) myofilament lattice spacing from the position of inner X-ray diffraction. Results: In all hearts, we did not detect incomplete relaxation at both heart rates. Increasing heart rate from 120 to 300bpm significantly decreased the intensity ratio reduction at the end-systole (0.92 ± 0.15 vs. 1.58 ± 0.17unitless, $p<0.01$) suggesting a smaller amount of actin-myosin interaction, but did not change myofilament lattice spacing (36.2 ± 0.5 vs. 36.7 ± 0.5 nm, NS). Conclusion: Increasing HR reduces the actin-myosin interaction without causing incomplete relaxation, indicating intact intracellular Ca^{2+} handling. These results may derive from shortening of Ca^{2+}-myofilament interaction.

Key-Words: - X-ray diffraction, whole heart, heart rate, left ventricle, actin, myosin

1 Introduction

Although cardiac muscles work with huge amount of actin and myosin interactions, i.e., crossbridge formation/dissociation dynamics, it is impossible to directly observe how crossbridge dynamics correlates with the left ventricular performance. Using third generation synchrotron radiation, we were able to observe real time crossbridge dynamics of twitch contraction in papillary muscles by x-ray diffraction analysis.

X-ray diffraction from a papillary muscle shows two pairs of spots. These diffraction spots are perpendicular to the longitudinal axis of papillary muscle. An inner pair of spots is the (1,0) equatorial reflection and corresponds to the lattice plane consisted of myosin-fibres. An outer pair is the (1,1) equatorial reflection and corresponds to the plane that consists of myosin and actin-fibres. Thus the intensity ratio of (1,1) over (1,0) equatorial reflections ($I_{(1,0)/(1,1)}$) correlates with the location of myosin heads in the filament lattice, i.e., crossbridge dynamics.

Recently we succeeded to extend the x-ray diffraction analysis technique to the beating rat left ventricles (LV) and to reveal the crossbridge formation/dissociation dynamics in the physiological states [1].

2 Materials and Methods

2.1 Surgical Preparations

Male Wistar rats (10 weeks old, n=8) were deeply anesthetized with diethyl ether. Midsternotomy was performed and the heart was quickly excised and submerged in oxygenated and warmed Tyrode's solution (136mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, and 5.0 mM HEPES, pH 7.4 at 37°C) containing 20 mM 2,3-butadione monoxime (BDM) and 20 U/ml of heparin. The severed end of the ascending aorta was cannulated with 18-gauge blunted needle that was connected to a Langendorff perfusion apparatus. The heart was perfused with the Tyrode's solution containing 20 mM BDM without heparin to remove blood and stop beating. Perfusion pressure was set at ~100 mmHg.

Then the heart was perfused with the Tyrode's solution containing 2.2 mM CaCl2 without BDM for re-beating. After recovery of spontaneous beat at sinus rhythm, complete heart block was achieved by focal formalin injection (10%, 0.1 ml) into the atrioventricular nodal region. The left atrium was opened and a thin latex balloon attached to an end of a stiff polyethylene tube was inserted into the LV cavity. The balloon and the

tube were filled with water and connected to a pressure transducer (Life Kit, Nihon Koden, Tokyo, Japan) to measure LVP and a custom-made syringe pump to control LV volume. A pair of pacing electrodes was placed at the apical end of the right ventricle and the heart was paced at rate of 120, 240, and 300 beats per min. To reduce the motion of the heart with beating, the heart was kept in a custom-made heart holder with a window to make x-ray beam irradiate the LV free wall. The torsion-like movement of the heart was not observed during the time-resolved x-ray recording. Sets of images with 70 frames/sec were recorded every 0.20 mm horizontally along the equator of the heart [1].

2.2 X-ray diffraction technique

The experiments were made at the beamline BL40XU in the third generation synchrotron radiation facility SPring-8 (Harima, Hyogo, Japan). The x-ray energy was 15.0 keV. The x-ray flux was adjusted with an aluminum absorber to ~ 2 X 1012 photons/s. The beam size (full width at half maximum) at the specimen was 0.21 mm horizontally and 0.10 mm vertically. The x-ray detector was a beryllium-windowed x-ray image intensifier (V5445P, Hamamatsu Photonics, Hamamatsu, Japan) coupled to a fast CCD camera (C4880-80-24A, Hamamatsu Photonics, Hamamatsu, Japan). For recording images from a beating heart, the time resolution of the x-ray images was 15 ms and 70 frames were recorded. A data acquisition system with an analog-to-digital converter was used to record the LVP, the coronary perfusion pressure, stimulus pulses, and the frame timing signals that indicated the beginning of each frame at the sampling rate of 1 kHz.

2.3 Intensity Ratio

Integrated intensities of $I_{(1,0)}$ and $I_{(1,1)}$ of the deeper layer of myocardium were determined from the areas under the reflection profile after background subtraction. Intensity ratio ($I_{(1,0)/(1,1)}$) was used for evaluation of actin-myosin interaction rather than absolute reflection intensities of $I_{(1,0)}$ that is strongly affected by the changes in the amount of myofilament in which x-ray passed due to beating.

According to the orientation of the muscle fibers, the reflection appeared in different angles and formed arcs or a ring [1]. Thus, the diffraction pattern was divided into twelve 30° sectors in such a way that the vertical line runs at the center of the top and bottom sectors. The intensity in each sector was averaged along an arc to obtain radial intensity distribution. Because the X-ray diffraction shows the centrosymmetrical patterns, we added the radial intensity distributions of two opposing sectors and obtained the intensity profile. Then the profiles were fittid with a fourth-order polynominal function that simulated the background, and two Gaussian functions that simulated the (1, 0) and (1, 1) reflection peaks. The series of analysis mentioned above was performed by the custom made program on Lab-VIEW (National Instruments, Texas, U.S.A.).

2.4 Myofilament Lattice Spacing

The (1,0) spacing ($d_{(1,0)}$) was obtained from the position of the (1,0) reflection because of their reciprocal relation. This is the separation of the (1,0) lattice planes and equal to $\sqrt{3}/2$ of the distance between centers of nearest thick filaments. The end-diastolic $d_{(1,0)}$ was calculated and the lattice spacing was calibrated using the 14.3 nm meridional reflection from frog skeletal muscle.

3 Results and Discussion

Along with increasing heart rate as 120, 240, and 300 bpm, LVP significantly decreased (67 ± 19, 50 ± 17, and 45 ± 18 mmHg, respectively) and the minimum value of $I_{(1,0)/(1,1)}$ ($I_{(1,0)/(1,1)min}$) also significantly increased (0.92 ± 0.15, 1.21 ± 0.10, and 1.58 ± 0.17, respectively), indicating a significant decrease in the amount of actin-myosin interaction. Myofilament lattice spacing, $d_{(1,0)}$, did not show significant change with increased heart rate (36.2 ± 0.5, 36.4 ± 0.4, and 36.7 ± 0.5 nm, respectively).

In our previous study, we found that decreased left ventricular volume significantly increased $I_{(1,0)/(1,1)min}$ and decreased developed left ventricular pressure under physiological conditions. Increasing heart rate significantly reduced the developed left ventricular pressure and increased $I_{(1,0)/(1,1)min}$. Increasing heart rate can decrease intracellular pH [2]. Decreased intracellular pH can reduce the contraction force by attenuated myofilament Ca^{2+} sensitivity or cumulation of inorganic phosphate by preventing the myosin ATPase[3, 4]. However these studies have been performed in papillary muscle or skinned muscle. The beating whole heart with normal circulation would be hard to cumulate non-volatile acid and inorganic phosphate in cytosol.

The cardiac contraction requires the actin myosin interaction. This interaction is tightly controlled by the cytosolic Ca^{2+} concentration, which has a crucial interaction with troponin C. At the cardiac excitation, sarcoplasmic reticulum, the intracellular Ca^{2+} store, releases Ca^{2+} and significantly increased cytosol Ca^{2+} concentration. In rat ventricle, sarcoplasmic Ca^{2+} content is often relatively high, even at low stimulation frequencies. This may be due to in part to relatively high intracellular Na^{+} concentration which limits Ca extrusion via Na^{+}/ Ca^{2+}exchanger[5, 6]. Thus increasing frequency in rat usually causes little or no further increase in, or even a slight decrease in sarcoplasmic reticulum Ca^{2+} content [7]. This might be a mechanism of our present finding.

We should compare the amplitude and duration of intracellular Ca^{2+} transients with various heart rates in beating whole heart but it will be a further experiment.

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